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PARENTERAL NUTRITION

VII. Metabolic Studies on Puppies Infused With Fat Emulsions

George V. Mann, M.D., Robert P. Geyer, Ph.D., Donald M. Watkin, M.D., Richard L. Smythe, A.B., Dsai-chwen Dju, Ph.D., Norman Zamcheck, M.D., and Fredrick J. Stare, M.D.
Boston, Mass.

THE preparation of fat emulsions with high caloric concentrations for parenteral supplementation or replacement of oral feeding has been studied intermittently by various workers for over fifty years.[1-4] Reports have indicated varying conclusions as to the practicability of preparing an emulsion of fat that can be given intravenously for nutritional purposes. Work done in this laboratory over the past six years has shown that fat emulsions suitable for intravenous administration can be prepared and that the calories in such preparations are available for energy requirements when the emulsions are given intravenously.[20]

The experiments described in this paper were undertaken in an effort to study the effect of intravenous fat emulsions upon growth, nitrogen balance, and selected biochemical and hematologic functions. Two litters of puppies were used to determine: (1) whether young and rapidly growing animals, with a consequently high caloric requirement, can utilize an emulsion of fat given intravenously; (2) whether daily infusion in large amounts (1.5 to 10.0 Gm. of fat per kilogram of body weight) of the emulsion now used in this laboratory* will lead to either histologic, hematologic, or metabolic injury.

EXPERIMENTAL

The puppies were housed in an air-conditioned room in individual metabolism cages permitting accurate collection of urine and feces. They were weighed daily. Nitrogen balance studies were conducted on a three-day interval scheme. The animals were maintained three days on a new regime and the nitrogen intake and output were then measured for each of three consecutive days and the balances were computed for each dog. Nitrogen determinations on diet and excreta were done by the macro-Kjeldahl method.

Hematologic studies were done on blood collected in a dry syringe from a foreleg vein and transferred to a tube containing balanced oxalate. Hemoglobin determinations were done by a direct photometric method.[6] During infusion periods, blood was drawn for hematologic and chemical studies just before the start of an infusion; this was usually twenty-four hours after the last infusion.

From the Department of Nutrition, Harvard School of Public Health, and the Departments of Biological Chemistry and Legal Medicine, Harvard Medical School.

This research was supported in part by grants-in-aid from the Williams and Waterman Fund of Research Corporation, New York, N. Y., the National Dairy Council, Chicago, Ill., the Upjohn Company, Kalamazoo, Mich., the Nutrition Foundation, Inc., New York, N. Y., and the Milbank Memorial Fund, New York, N. Y.

Received for publication, Aug. 17, 1948.

*Emulsion No. 35, composition in grams: coconut oil, 300; phosphatide fraction BF2, 30; dextrose, 35; water, 634; for details of preparation see reference 5.

Total serum protein and serum albumin were determined by the method of Howe,[7] serum nonprotein nitrogen by the nesslerization procedure of Koch and McMeekin,[8] and serum bilirubin by the diazotization procedure of Malloy and Evelyn.[9] Serum total and free cholesterol determinations were done by the method of Schoenheimer and Sperry.[10]

Bromsulfalein excretion was determined by the method previously described by McKibbin[11] and plasma prothrombin by the method of Link.[12] Plasma volumes were determined by the method of Hopper and co-workers[13] and red blood cell fragility by the method of Wintrobe[14] or Parpart.[15] Fecal lipids were determined as follows: Collections of the twenty-four hour feces for each dog were mixed in a Waring blendor with the additions of 95 per cent ethyl alcohol and concentrated hydrochloric acid until acid to Congo red. An aliquot of the mixture was dried in vacuo and extracted with dry chloroform for twenty-four hours in a Soxhlet extractor. The chloroform was then removed in vacuo, the residual fat taken up in low-boiling (40 to 60° C.) petroleum ether and filtered into tared beakers. The ether was removed and the chloroform-extractable, petroleum ether-soluble lipid determined by weighing.

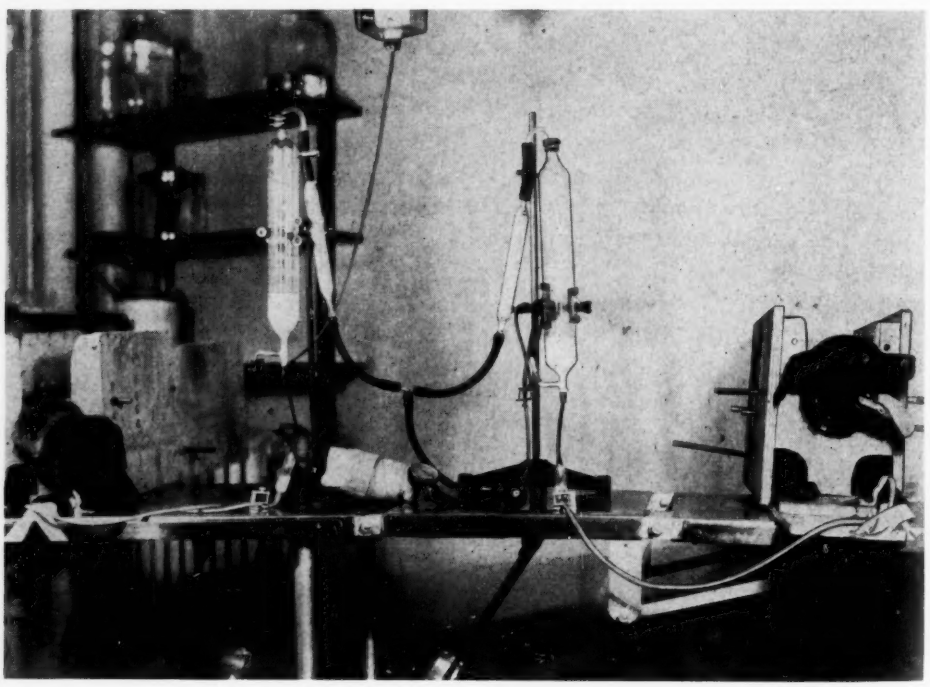

Fig. 1.—A photograph of the apparatus used for infusion of animals.

Infusions of fat emulsion were carried out by placing the animal in an adjustable animal box in an upright position. A foreleg was secured with adhesive tape and after preparing the area for injection, the needle (No. 20 or No. 22) attached to the infusion set was inserted into a vein on the dorsum of the leg. When the needle had been taped securely in place the infusion could run for several hours without discomfort to the animal. For accurate measurement of infusion rates we found it advantageous to use an infusion set consisting of a graduated cylinder as a reservoir, a Murphy drip bulb, and a closed system with positive air pressure maintained by an attached blood pressure cuff and aneroid manometer (Fig. 1).

The composition of the various diets fed the puppies in these studies is shown in Table I.

TABLE I. COMPOSITION OF DIETS USED FOR EXPERIMENTS 1 AND 2

DIET	D1 (GM. %)	D2 (GM. %)	GAINES DOG MEAL†	(GM. %)
Casein (crude)	20	10	Protein	25.1
Liver extract, Wilson's 1-20	2	2	Ash	8.6
Yeast, Anheuser-Busch, strain K	10	10	Fat	6.6
Gaines Dog Meal	0	78	Fiber	2.9
Dextrose	65	0	N.F.E.	49.6
Salt mixture IV*	3	0	Moisture	7.2

*J. Biol. Chem. **138**: 459, 1941.
†Manufacturer's analysis.

Experiment 1.—A litter of eight Labrador retriever puppies reared in this laboratory was weaned at 6 weeks of age and during two weeks were gradually transferred to diet D1. The animals were fed ad libitum; the daily food intake was measured; and nitrogen balances were determined at intervals.

After an initial control period, four puppies (Nos. 905, 906, 907, and 909) were started on daily infusions of a 30 per cent coconut oil emulsion stabilized with a 3 per cent phosphatide preparation—Emulsion No. 35.[5] Two pups, Nos. 904 and 908, were kept as control animals and fed ration D1 ad libitum.

The initial infusions were given rapidly at a rate of 2 to 4 ml. per minute and led to two immediate toxic reactions. On the first day of infusions the animals showed evidence of vasomotor collapse with pale mucous membranes and sometimes with drowsiness and defecation. This reaction occurred after no more than 25 ml. of the emulsion had been given. After five to ten minutes, with the infusions continuing at the initial rate, the animals recovered. The reaction did not reoccur on successive days. The second complication of immediate importance was the occurrence of vomiting and anorexia. Vomiting appeared either as a result of rapid infusion or after long-continued infusion at a slow rate. Since appetite is determined in part by caloric requirement, the infusion of such a large amount of fat (10 Gm. of fat per kilogram of body weight) to supply calories would be expected to decrease the oral intake of food to the point of serious limitation of protein, vitamins, and minerals. These limitations would in turn aggravate the anorexia and if continued for any time would give rise to serious deficiency complications. When the limitation of oral intake became apparent (after five to seven days) the infusions were stopped for five days, and the animals, including the two control pups, were transferred to a diet of Gaines Dog Meal to allow them to regain nutritional equilibrium. Infusions in the experimental animals were commenced again at a rate of 3 to 5 ml. per minute but in a total quantity to supply only 1.5 Gm. of fat per kilogram of body weight per day. This was well tolerated. After six days the quantity of emulsion infused was increased to 3 Gm. of fat per kilogram. At this level of infusion growth was good and the animals were in positive nitrogen balance while receiving approximately 20 per cent of their caloric requirement as intravenous fat. Figs. 2, 3, 4, and 5 show the data on oral food consumption, infusion of fat, growth, hematologic data, and nitrogen balance obtained on these four puppies. The hematologic data reveal a moderate but significant fall in hemoglobin, red blood cell count,

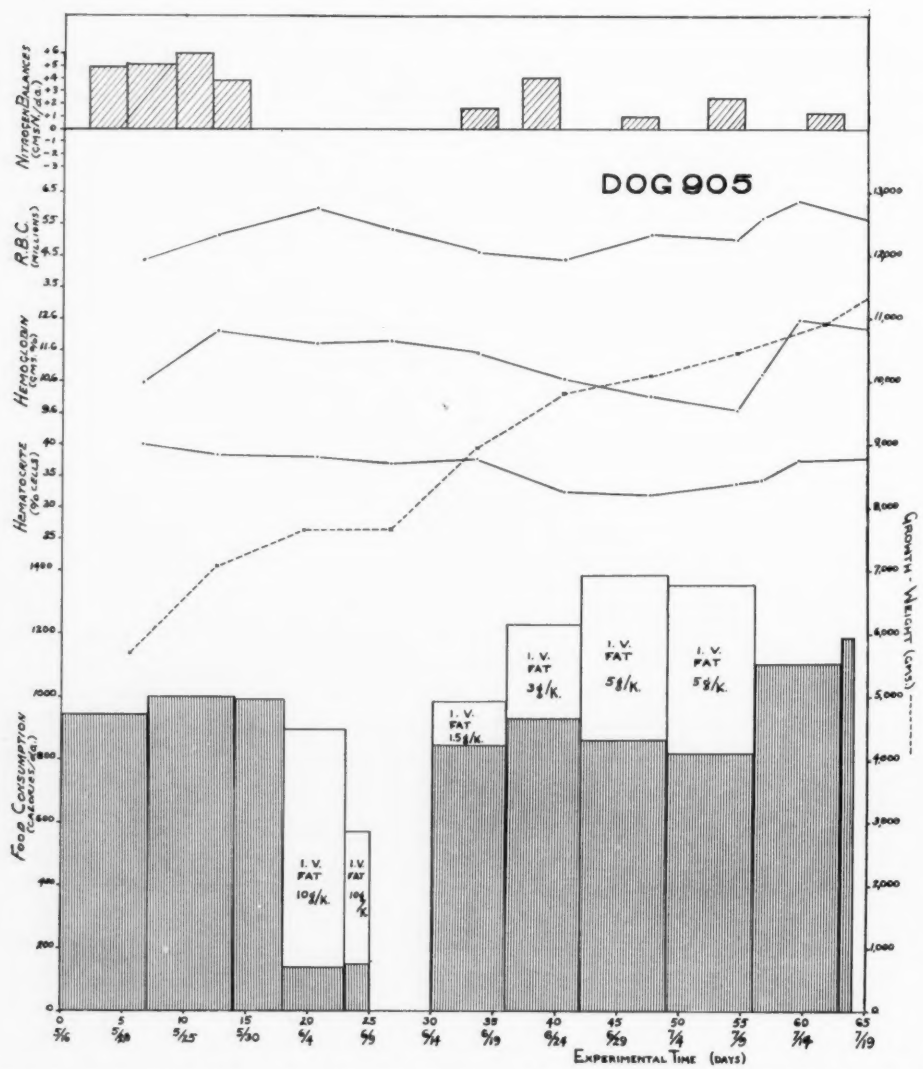

Fig. 2.—Data obtained from Dog No. 905, Experiment 1—infusion of 30 per cent fat emulsion.

and hematocrit. Similar blood changes have been reported from this laboratory by Collins and co-workers.[16] It is of interest to note that the anemia produced is self-limited even though infusions continue. The data presented on Pup No. 909 in Fig. 5 particularly emphasizes this point.

The puppies received 3 Gm. of fat per kilogram of body weight per day for six days and then the amount of infused fat was increased to 5 Gm. of fat per kilogram. At this higher level the results obtained were not so uniform. Puppies 905 (Fig. 2), 906 (Fig. 3), and 907 (Fig. 4) grew well and remained in positive nitrogen balance, but Puppy 909 (Fig. 5) grew poorly and was for a time in negative nitrogen balance. After twenty-one days on this infusion level of 5 Gm. of fat per kilogram of body weight, Pup 909 was

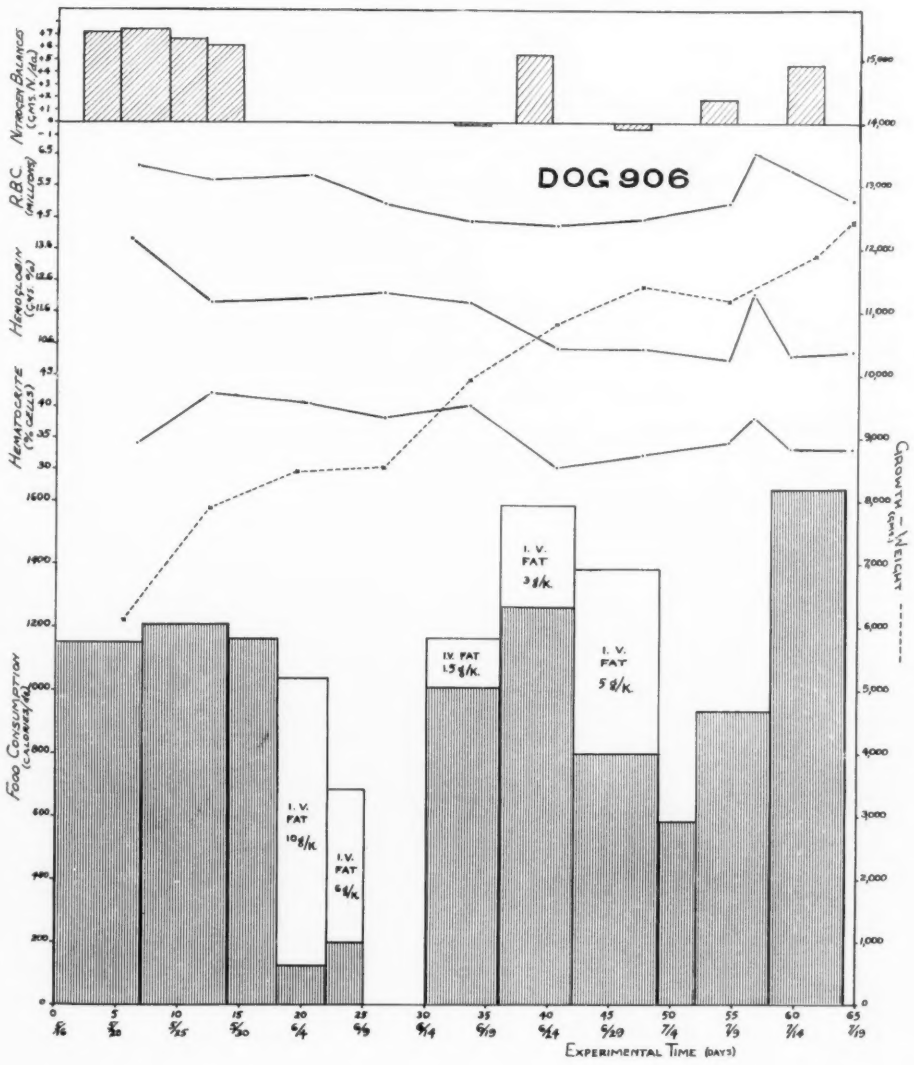

Fig. 3.—Data obtained from Dog No. 906, Experiment 1—infusion of 30 per cent fat emulsion.

again given 10 Gm. of fat per kilogram per day and continued on this regime for an additional twenty-four days with the object of determining the effect of continued large infusions of fat. At this extremely high level of fat infusion, the blood lipid level remained high even twenty-four hours after the last infusion, indicating that the infusions were surpassing the utilization and storage rates. There was occasional vomiting during or immediately after the infusions. Oral intake of food remained essentially constant for the first two weeks following this high level of fat and then progressively diminished. It will be noted that there was a steady gain of weight, with an increasingly positive nitrogen balance, until the seventy-fifth day of the experiment. After

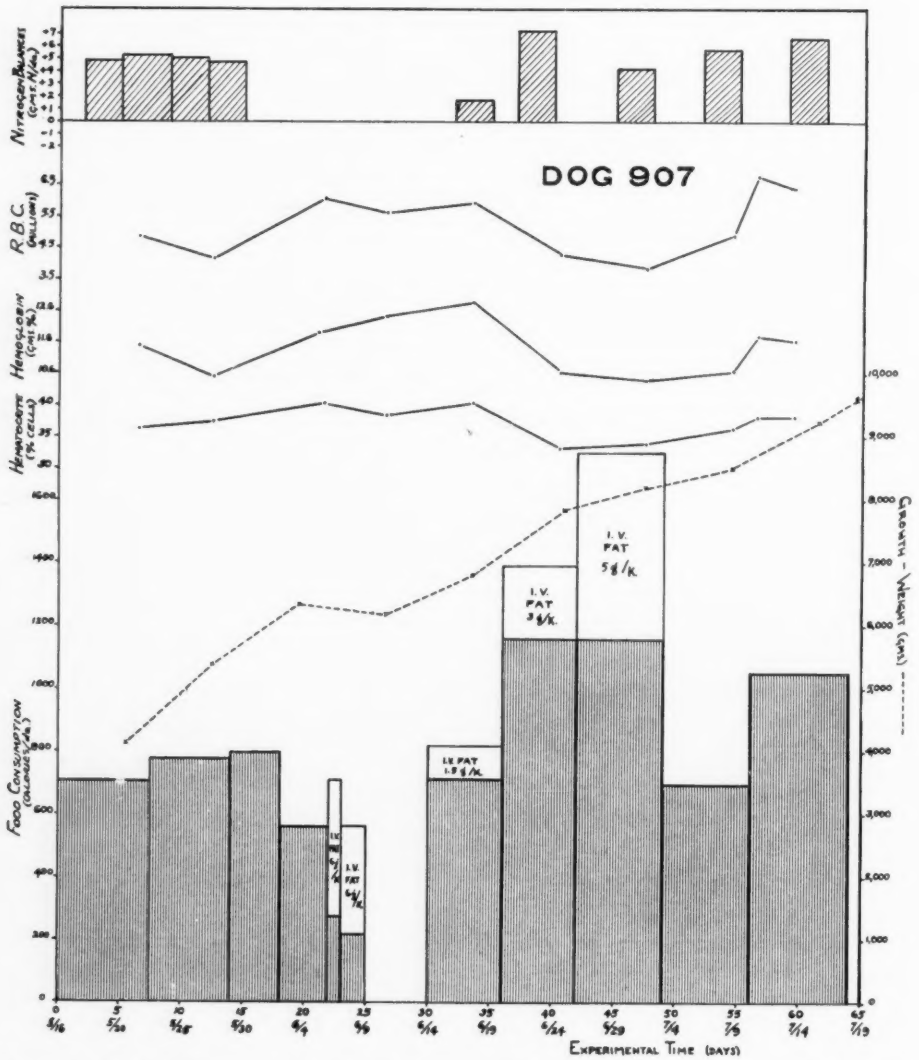

Fig. 4.—Data obtained from Dog No. 907, Experiment 1—infusion of 30 per cent fat emulsion.

this time, the oral intake fell to inadequate levels, the animal became thin and gaunt and died on the ninety-first experimental day. Over the course of the experiment, this animal had received 3,030 Gm. of fat by vein. The terminal rise in blood values was probably due to dehydration and hemoconcentration. The precise cause of this dog's death is not known, though the anorexia and inadequate intake of essential nutrients were no doubt important factors. The loss of fat tolerance as indicated by progressive abnormality of tolerance curves may be of significance.

The two control pups (Nos. 904 and 908) which had been maintained on a diet of Gaines Dog Meal grew well, showed a normal blood picture, and except for occasional respiratory infections, appeared in good health.

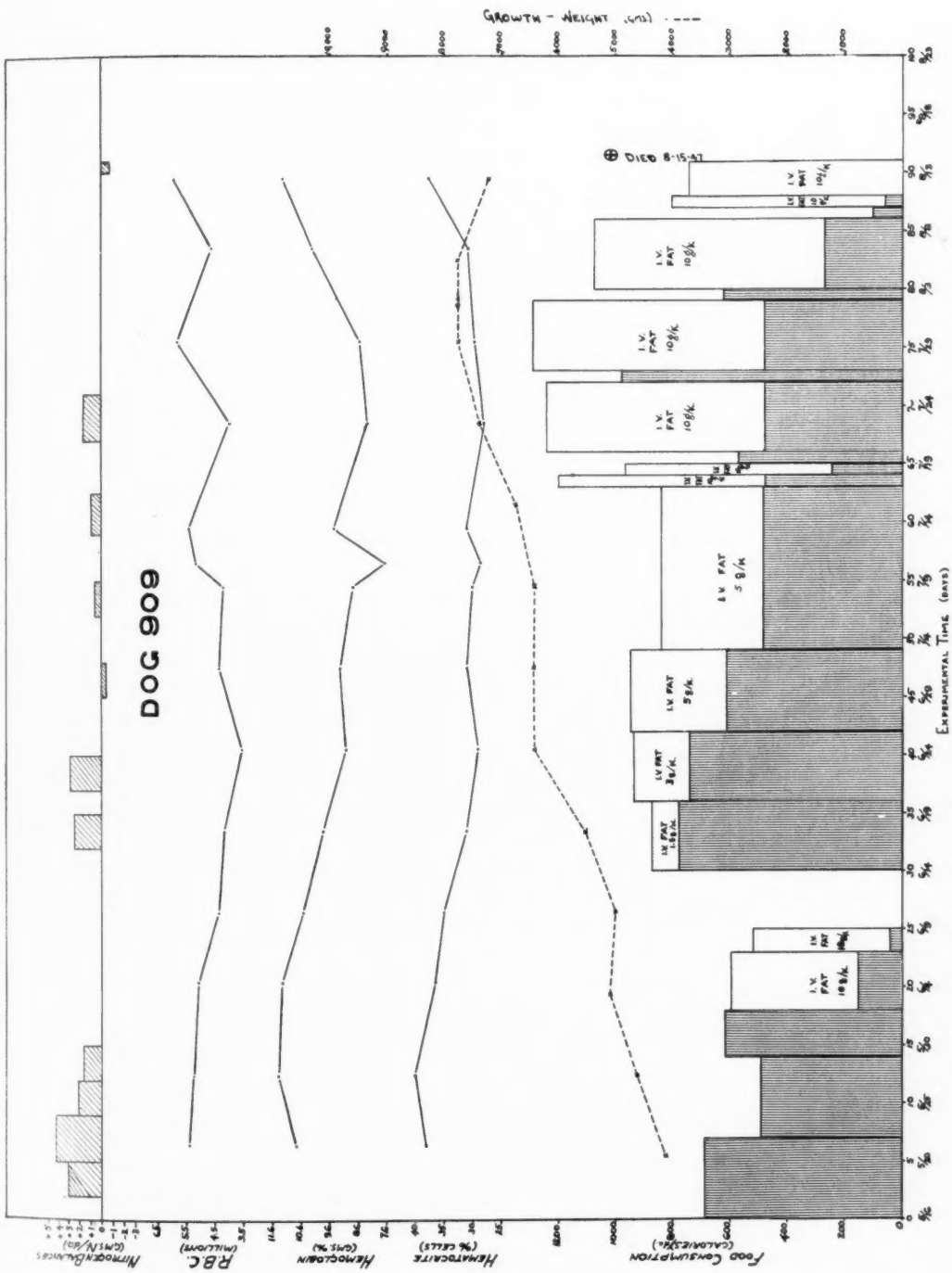

Fig. 5.—Data obtained from Dog No. 909, Experiment 1—infusion of 30 per cent fat emulsion.

Pathology of Animals in Experiment 1: Table II indicates the relationship of the infusions to the time of autopsy of this group of animals. At postmortem the animals in Experiment 1 showed no gross changes. Microscopic examination of stained sections revealed that the lungs of animals 905 and 907,

TABLE II. SUMMARY OF FAT INFUSIONS IN EXPERIMENT 1

DOG	DAYS INFUSED	TOTAL FAT INFUSED (GM.)	DAYS AFTER LAST INFUSION ANIMAL SACRIFICED	COMMENT
904	0	0	---	Control animal
908	0	0	---	Control animal
907	22	647	18	Tolerance good
906	26	1,225	110	Tolerance good
905	33	1,466	20	Tolerance good
909	63	3,030	5 hr.	Animal died

infused for thirty-three and twenty-two days respectively, contained occasional accumulations of multinucleated giant cells. These were graded 1+ according to the criteria previously outlined.[5] No granulomatous lesions were found. Fat stains of the liver were not remarkable. The bone marrow showed a moderate degree of hyperplasia. The remaining organs were normal. Animal 909 showed evidence of terminal pneumonia with peribronchial infiltration of inflammatory cells, but neither granulomatous nor giant cell-containing lesions were found. The control animal (No. 904) sacrificed at this time showed similar evidence of pulmonary infection suggesting a viral disease. Infusion animal No. 906 was not sacrificed until one hundred and ten days after the last infusion. The remaining control pup (No. 908) was sacrificed at that time. Neither animal showed any evidence of histologic changes.

Experiment 2.—With the experience of the preceding study a second experiment was designed to accomplish two purposes: (1) to avoid the immediate toxic reactions and anorexia complicating the first experiment by more judicious adjustment of the rate and amount of infusion and composition of the diet; (2) to study several metabolic functions of the animals in order to determine the effects of both the complete emulsion (Emulsion 35[5]) and the phosphatide emulsifier BF2[5] alone.

A litter of eight mongrel puppies was placed on diet D2 (Table I) at 16 weeks of age. The animal procedures and nitrogen collections were carried out as described for the preceding experiment. In addition to hematologic studies, the following determinations were made at regular intervals: serum total protein and albumin, serum total and free cholesterol, serum nonprotein nitrogen, plasma volume, bromsulfalein clearance, serum bilirubin, and plasma prothrombin concentration.

The eight animals were divided into four groups of two animals each as indicated in Table III. Animals I and II were infused with a 30 per cent coconut oil emulsion stabilized with 3 per cent of a phosphatide fraction (Emulsion 35): animals III and VI, with an emulsion of 3 per cent phosphatide fraction BF2[5] without added fat; animals IV and V were not infused but were pair-fed with animals I and II respectively. Animals VII and VIII served

as controls, were not infused, and were fed diet D2 ad libitum. The infusions were given at a rate of 3 to 5 ml. per minute, and animals I and II which received the fat emulsion obtained a total of 2 Gm. of fat per kilogram of body weight per day from this source for the first two days, 3 Gm. of fat per kilogram for the next seventeen days, and 5 Gm. of fat per kilogram for the last twelve days.

TABLE III. SUMMARY OF FAT INFUSIONS IN EXPERIMENT 2

DOG	INFUSION	ORAL FOOD	INFUSION TIME (DAYS)	TOTAL COCONUT OIL INFUSED (GM.)	TOTAL PHOSPHO-LIPID BF2 (GM.)	TIME AFTER LAST INFUSION SACRIFICED (DAYS)
I	30% fat emulsion	Diet D2 ad libitum	31	1,706	171	16
II	30% fat emulsion	Diet D2 ad libitum	31	1,386	139	16
III	3% BF2	Diet D2 ad libitum	31	0	106	16
VI	3% BF2	Diet D2 ad libitum	31	0	142	16
IV	0	Pair-fed with Dog I	4	292	2.92	9
V	0	Pair-fed with Dog II	4	292	2.92	5 hr.
VII	0	Ad libitum control	0	0	0	0
VIII	0	Ad libitum control	0	0	0	0

The course of this experiment is charted in Figs. 6, 7, 8, and 9. Fig. 6 presents the data obtained on animals I and II which were infused with the 30 per cent of fat emulsion. The data emphasize again adjustment of appetite and oral food intake to maintain a constant total caloric intake. These two puppies grew well if not better than the ad libitum fed control animals (animals VII and VIII, Fig. 9) although they obtained 25 to 30 per cent of their total calories from intravenous fat. Fig. 7 shows the data on animals III and VI and it is seen that the phosphatide material BF2 alone had little, if any, effect upon appetite or growth.

Nitrogen assimilation was comparable in the infused dogs with that of the control, noninfused animals. In sharp contrast, Dogs IV and V, pair-fed with Dogs I and II, were barely able to maintain their body weight and nitrogen equilibrium (Fig. 8). They were, of course, receiving fewer calories to the extent of the calories furnished by the parenteral fat to Puppies I and II. The growth curves of each pair of animals in this experiment have been averaged and are all presented for comparison in Fig. 10.

Since Puppies IV and V were behind in growth, it was decided to give them large infusions of fat to see whether there would be a prompt gain in weight. Accordingly, on the fifty-second day of the experiment a series of four infusions of a 30 per cent fat emulsion amounting to 10 Gm. of fat per kilogram of body weight were given. Unfortunately, this emulsion was prepared by a different procedure and proved to be unstable in vivo. As Figs. 8 and 11 indicate, it was immediately toxic. Because of the toxic reactions of this poor batch of emulsion, animals IV and V are excluded from the discussion of post-mortem changes.

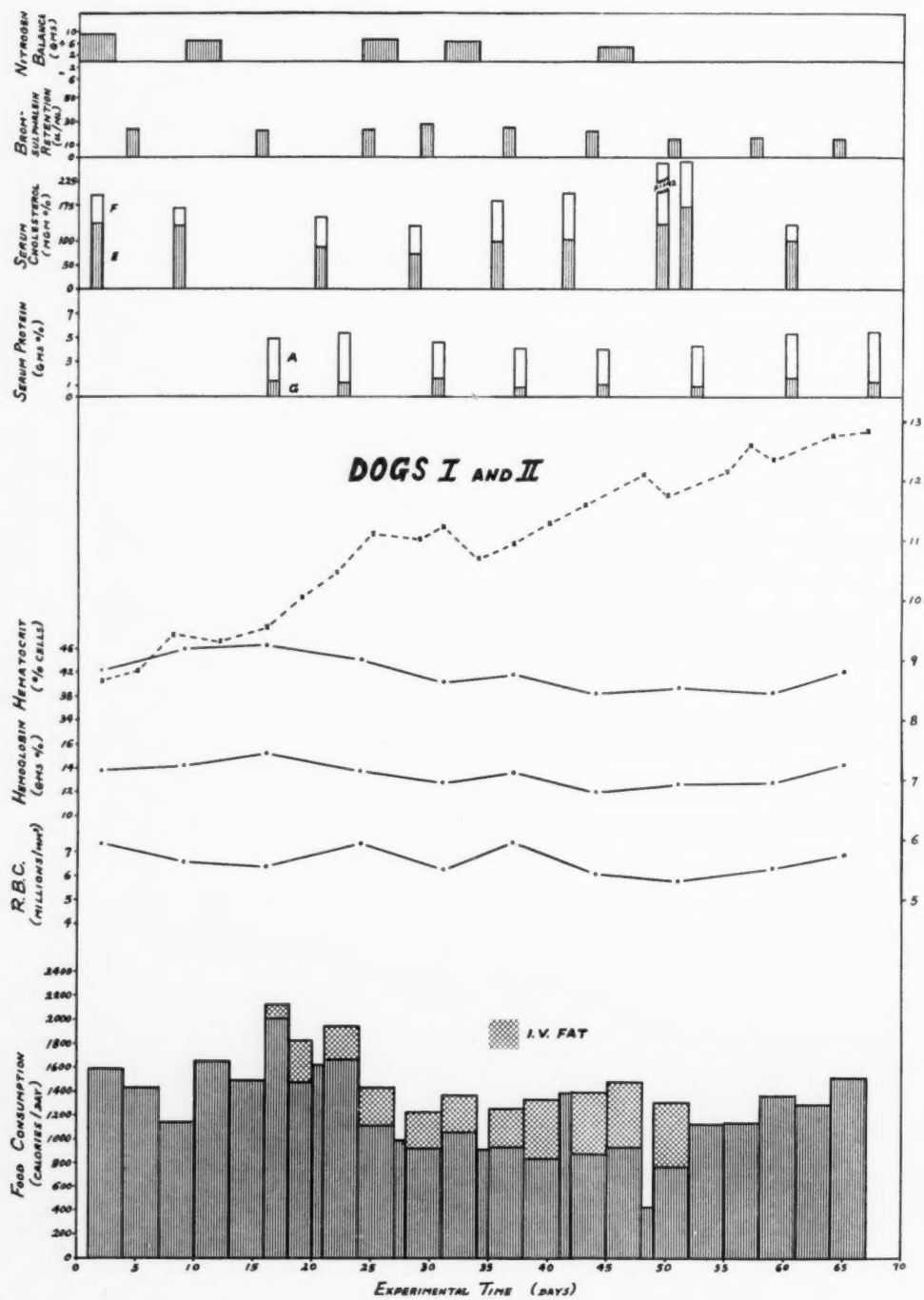

Fig. 6.—Data obtained from Dogs I and II, Experiment 2—infusion of 30 per cent fat emulsion

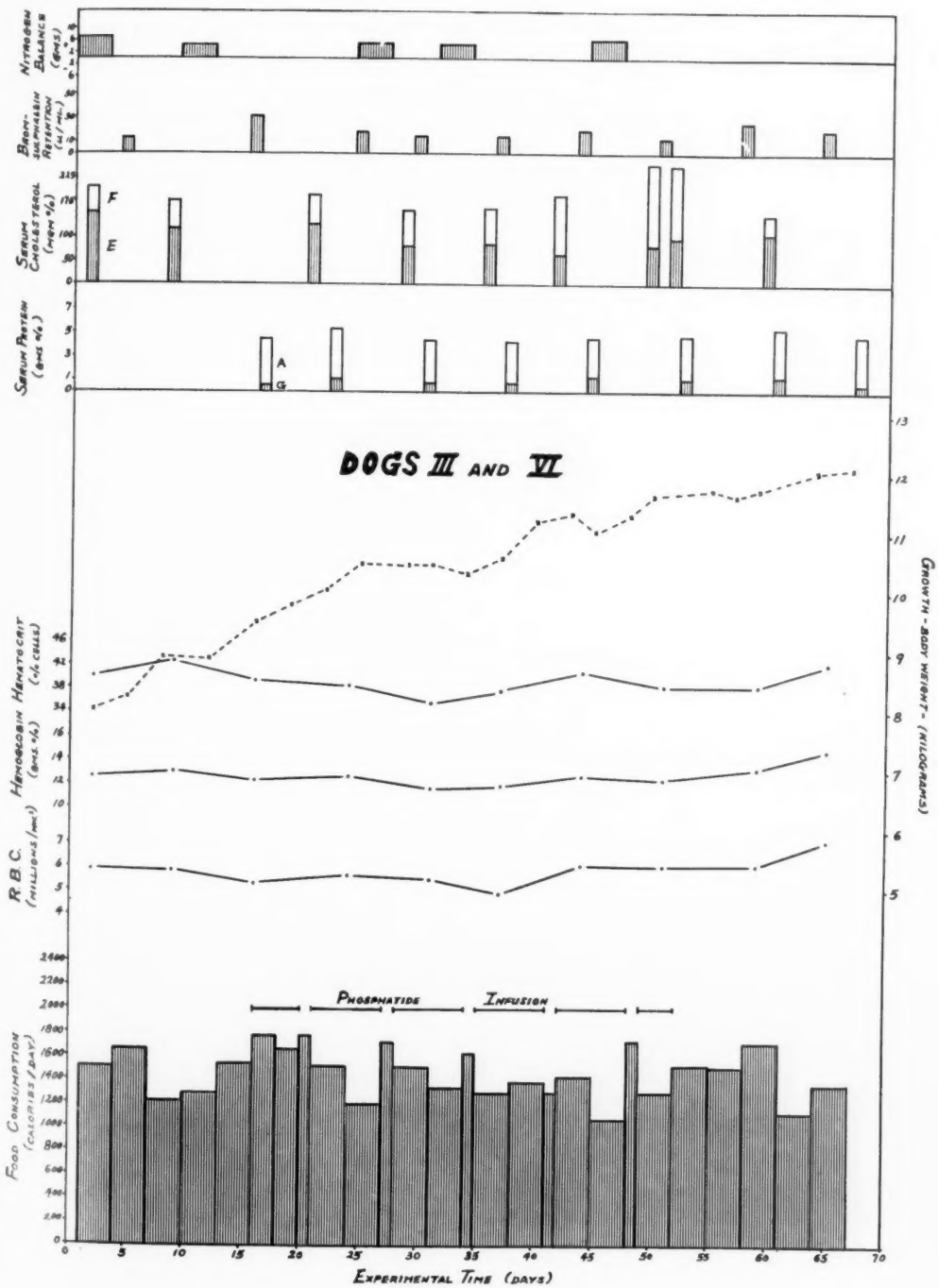

Fig. 7.—Data obtained from Dogs III and VI, Experiment 2—infusion of phosphatide stabilizer without added fat.

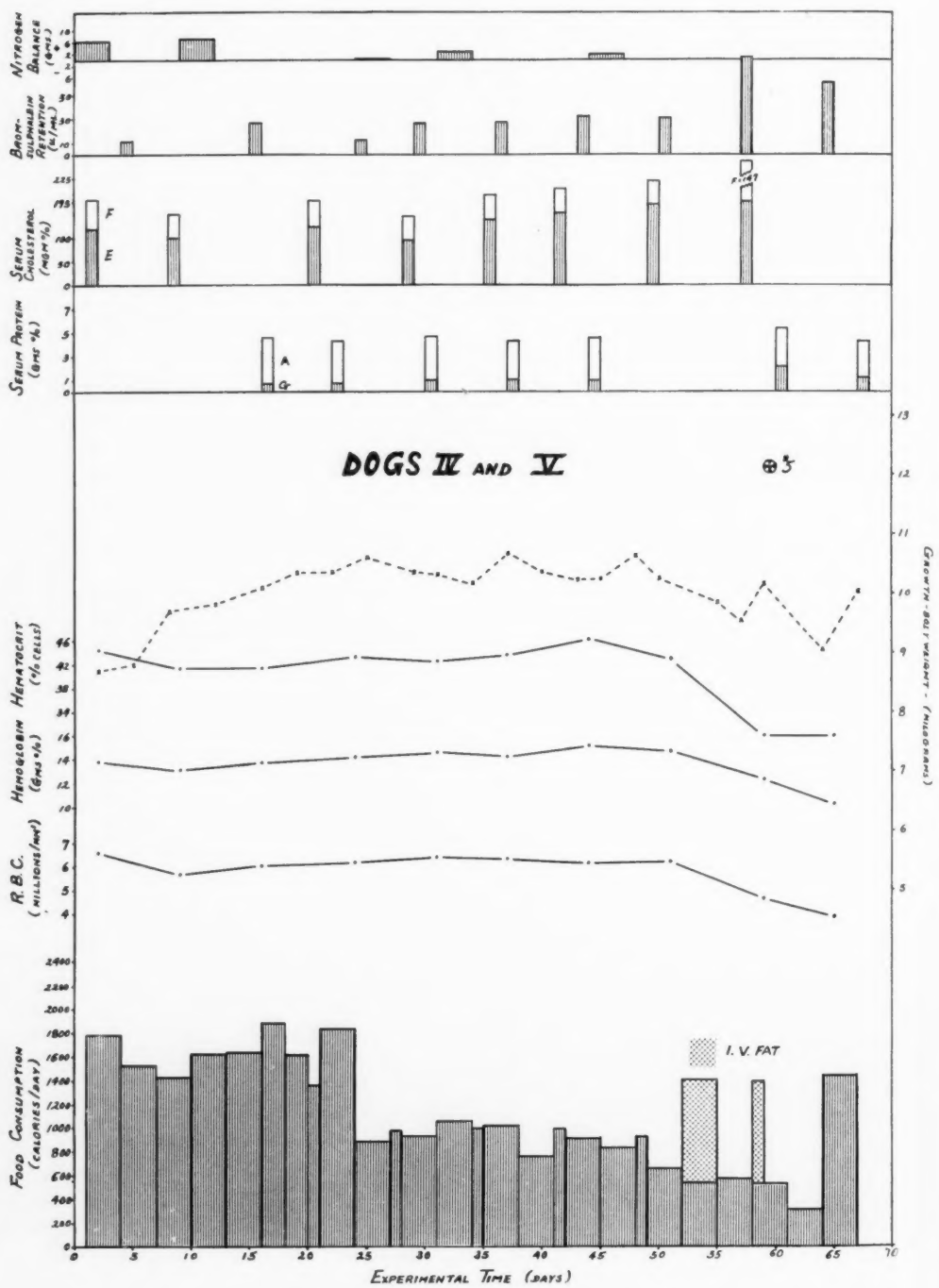

Fig. 8.—Data obtained from Dogs IV and V, Experiment 2—control animals, pair-fed with Dogs I and II, no infusion.

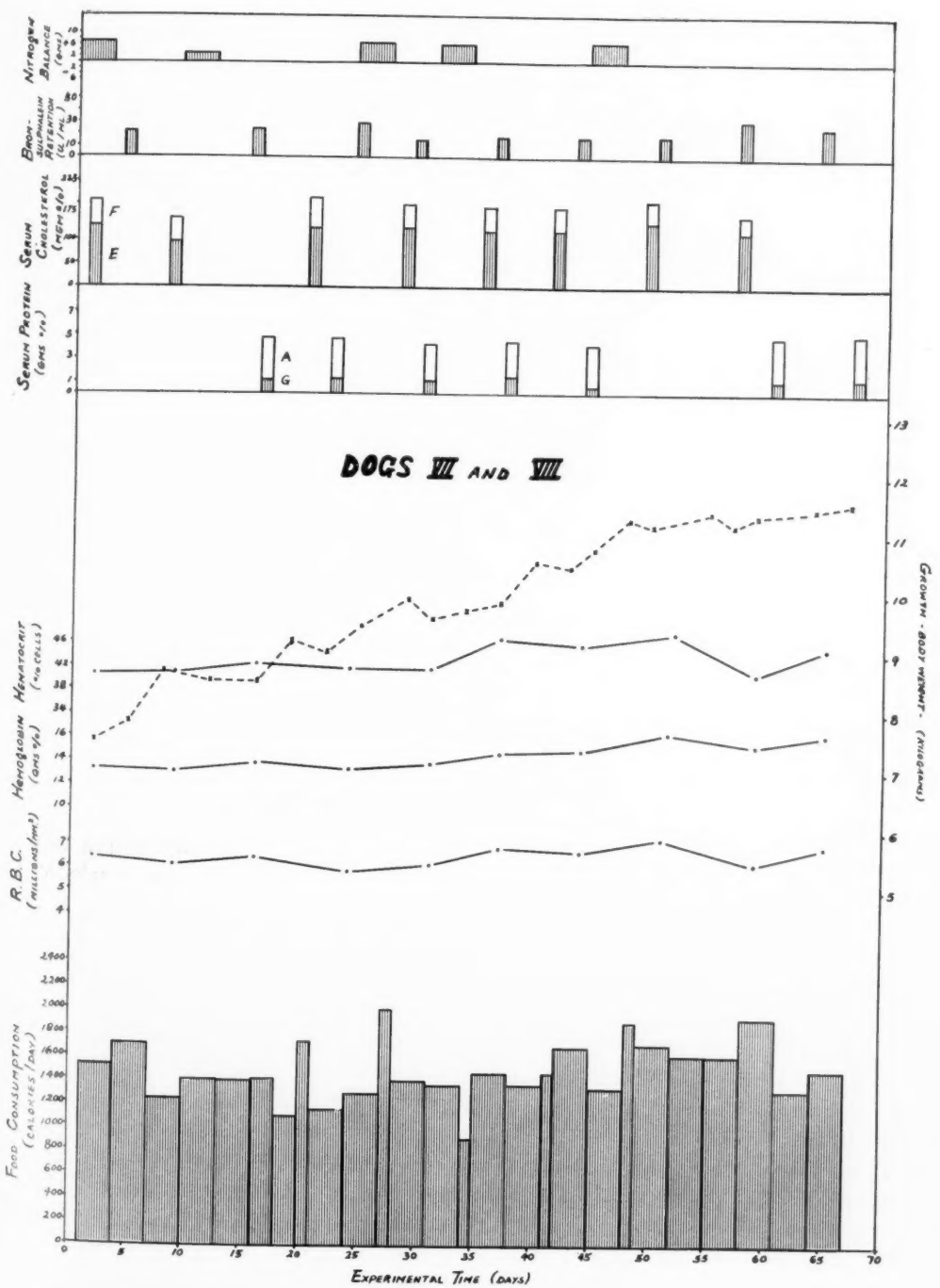

Fig. 9.—Data obtained from Dogs VII and VIII. Experiment 2—control animals, fed ad libitum, no infusion.

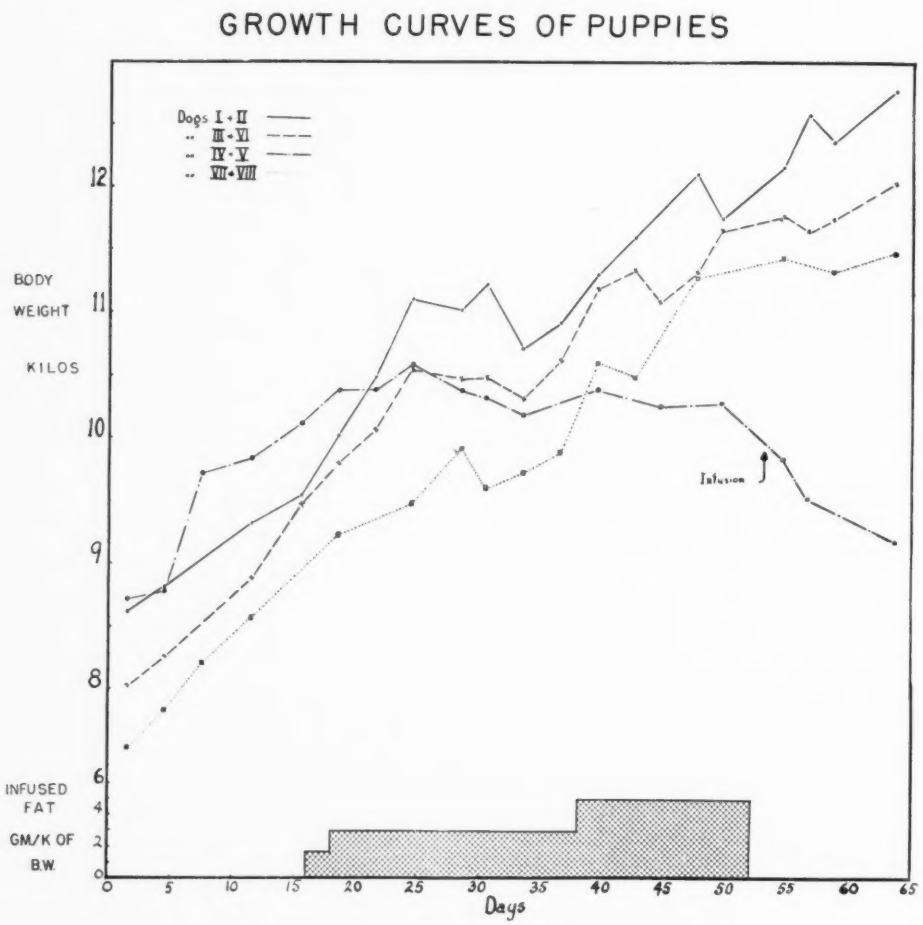

Fig. 10.—Growth curves of all animals in Experiment 2.

Hematologic studies revealed a small but significant and selflimiting fall of hemoglobin, hematocrit, and red blood count during the first two weeks of the infusion period. This change was of the same magnitude in the animals receiving the phosphatide BF2 alone as in those receiving the fat emulsion. Serum bilirubin determinations during this period did not reveal evidences of increased blood destruction.

Determinations of red blood cell fragility revealed consistently decreased resistance of the infused animals' blood cells to hypotonic saline; this seemed to be caused by the phosphatide emulsifier rather than by the fat itself since the effect was no greater in the dogs receiving the coconut oil emulsion than in those receiving only the phosphatide. The mechanism of this effect has not been established.

Neither the serum protein concentration nor the total circulating serum protein calculated from plasma volumes was significantly altered. Serum albumin concentration and total circulating serum albumin were not significantly changed. The serum nonprotein nitrogen remained within normal limits.

An interesting and perhaps significant finding detected in the infused animals was a striking increase in the proportion of free serum cholesterol. This increase became significant two weeks after the start of the infusions and it was more marked in the animals receiving only the phosphatide stabilizer. One week after cessation of infusion, the ratio of free to total cholesterol had returned to normal. The serum cholesterol changes in Puppies

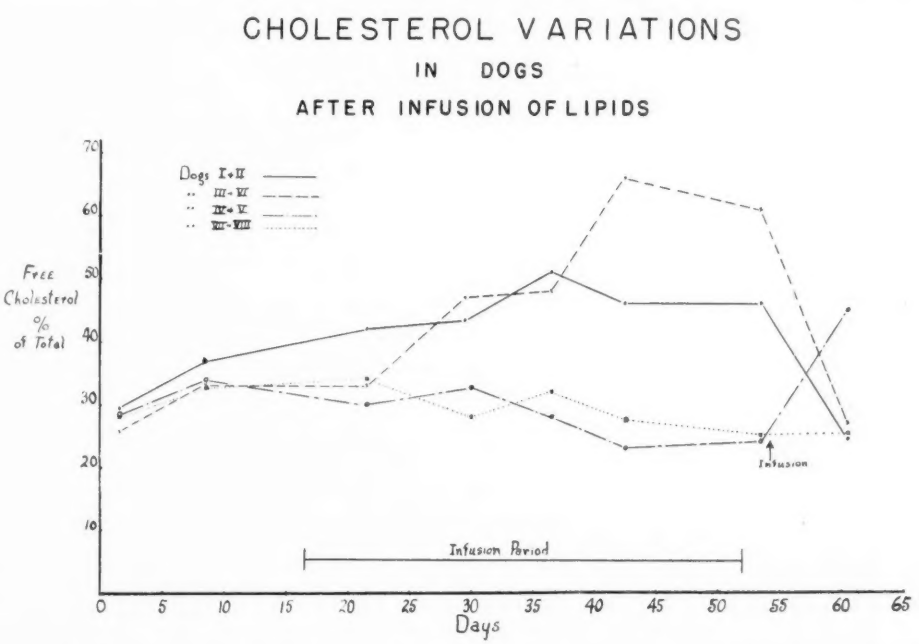

Fig. 11.—Cholesterol variations in all dogs in Experiment 2; Dogs I and II infused with 30 per cent fat emulsion, Dogs III and VI infused with phosphatide stabilizer without added fat, Dogs IV, V, VII, and VIII not infused.

IV and V, which received four infusions of a poor batch of emulsion beginning on the fifty-second day of the experiment, are of interest. A week after the last of these infusions the ratio of free to total cholesterol had risen from a normal range of 0.25-0.33, to 0.46. There was a small increase of total serum cholesterol, and there was a prompt increase of bromsulfalein retention. Fig. 11 is a graphic representation of the cholesterol data.

Except in Dogs IV and V after they had received the unstable and toxic emulsion, the liver function, as determined by serial bromsulfalein tests, revealed no abnormality to correlate with the serum cholesterol. Plasma prothrombin determinations at the close of the infusion period were normal.

The possibility of an increased fecal excretion of fat by the animals receiving fat emulsions was considered and hence twenty-four hour stool specimens were analyzed for total lipids. Table IV illustrates the fecal lipid excretion of the animals for an average period. It is seen that fecal lipids are not increased during the infusion of fat. Urinary studies showed that the urine contained insignificant amounts of lipids.

TABLE IV. FECAL LIPID EXCRETION DURING FAT INFUSION IN EXPERIMENT 2

DOG	INFUSION	AVERAGE 24-HOUR FECAL LIPID EXCRETION (GM.)
I	Fat, 5 Gm./kg.	3.53
II	Fat, 5 Gm./kg.	
III	Phosphatide, 0.5 Gm./kg.	2.71
VI	Phosphatide, 0.5 Gm./kg.	
IV	0	1.65
V	0	
VII	0	4.06
VIII	0	

Pathology of Animals in Experiment 2: The animals in Experiment 2 were sacrificed for post-mortem study as indicated in Table III. The gross appearance of the organs and tissues was normal. Table V indicates the organ weights at autopsy. These were not unusual except for the spleens of animals III and VI which were significantly enlarged. Sections of the tissues were preserved in 10 per cent formalin for histologic study and other sections of the lungs, heart muscle, liver, spleen, pancreas, and kidneys were removed and immediately frozen at −20° C. and stored at this temperature for lipid analyses.

TABLE V. ORGAN WEIGHTS OF ANIMALS IN EXPERIMENT 2 AT AUTOPSY

DOG	MATERIAL INFUSED	INFUSION DURATION (DAYS)	INTERVAL AFTER INFUSION BEFORE AUTOPSY (DAYS)	LUNGS (GM.)		HEART (GM.)	LIVER (GM.)	SPLEEN (GM.)	PANCREAS (GM.)	KIDNEYS (GM.)	
				L	R					L	R
I	Fat emulsion	31	16	40	65	95	530	75	25	30	30
II	Fat emulsion	31	16	45	65	115	560	65	29	37	37
III	Phosphatide fraction BF2	31	16	18	45	65	380	80	20	25	25
VI	Phosphatide fraction BF2	31	16	42	62	100	500	85	36	42	37
VII	0	0	0	35	52	97	385	37	32	33	33
VIII	0	0	0	37	54	78	371	24	23	26	28

Microscopic examination of sections of the lungs of the infused animals showed them to be normal. Sections stained with sudan III revealed no unusual fat deposition. Sections of heart muscle were normal as were the sections of aorta. No intimal lipid deposits could be demonstrated. The thymus and lymph nodes were not unusual and the adrenals appeared normal.

The spleens and livers of all infused animals showed an increase in pigment which appeared as brownish-black granules in the sinusoids and Kupffer cells. This pigment was more prominent in animals III and IV which had received the phosphatide stabilizer alone than in the animals receiving the emulsion of fat. All the infused animals were found to have occasional small focal accumulations of mononuclear cells in the liver; these consisted of four to ten cells negative for lipid by sudan stain. There were no multinucleated giant cells. There was no evidence of abnormal fat deposition in the liver.

The kidneys were found to be normal in all animals. The marrow, unfortunately, was not suitably fixed for adequate study.

The histologic changes following the infusions were thus of two types—both minimal: first, infused animals showed an increase of pigment deposition in the phagocytic cells of the liver and sinusoids of the spleen, and second, scattered, small, round cell infiltrations were found in the liver.

Lipid Analysis of Organs From Animals in Experiment 2: The frozen tissues were thawed and two portions of each organ were dissected free of adherent tissues. One portion was weighed wet, dried in an oven at 105° C. for

TABLE VI. LIPID CONTENT AND DISTRIBUTION IN THE VARIOUS ORGANS AFTER INFUSION

(All values expressed as grams per cent of dry weight; all values are means of duplicate determinations on tissues from two animals)

ORGAN	INFUSION	FAT EMULSION (30% COCONUT OIL)	PHOSPHATIDE FRACTION (3% BF2)	CONTROLS (NO INFUSION)
Liver	Total lipid	13.8	13.9	13.2
	Total fatty acid	7.69	8.06	5.05
	Phospholipid	9.14	8.59	7.80
	Neutral fat	2.58	3.41	2.46
	Total cholesterol	0.74	1.02	0.45
	Free cholesterol	0.66	0.83	0.30
	Ester cholesterol	0.08	0.19	0.15
Lung	Total lipid	15.1	14.5	17.4
	Total fatty acid	7.73	7.42	9.45
	Phospholipid	8.79	9.04	9.90
	Neutral fat	3.01	2.16	4.02
	Total cholesterol	2.02	2.14	1.85
	Free cholesterol	2.01	2.02	1.85
	Ester cholesterol	0.01	0.12	0.00
Spleen	Total lipid	10.1	6.70	10.6
	Total fatty acid	4.17	2.86	4.45
	Phospholipid	6.28	3.58	5.90
	Neutral fat	0.81	0.94	1.14
	Total cholesterol	1.50	1.29	1.70
	Free cholesterol	1.53	1.29	1.70
	Ester cholesterol	0.00	0.00	0.00
Heart	Total lipid	13.6	12.1	18.5
	Total fatty acid	7.95	6.59	11.1
	Phospholipid	9.35	8.59	9.20
	Neutral fat	2.20	1.80	5.99
	Total cholesterol	0.61	0.54	0.55
	Free cholesterol	0.52	0.54	0.45
	Ester cholesterol	0.09	0.00	0.10
Kidney	Total lipid	15.2	16.6	17.5
	Total fatty acid	8.78	8.18	10.4
	Phospholipid	11.1	11.7	12.7
	Neutral fat	2.59	1.50	3.69
	Total cholesterol	1.28	1.75	1.55
	Free cholesterol	1.28	1.63	1.50
	Ester cholesterol	0.00	0.12	0.05
Pancreas	Total lipid	13.6	12.9	15.2
	Total fatty acid	8.46	6.92	8.85
	Phospholipid	8.85	4.58	6.4
	Neutral fat	3.75	4.50	5.40
	Total cholesterol	0.90	1.02	1.10
	Free cholesterol	0.87	1.02	1.05
	Ester cholesterol	0.03	0.00	0.05

forty-eight hours to a constant weight, and the percentage dry matter calculated. Another similar portion was weighed and ground with three times its weight of anhydrous sodium sulfate until a fine, pink powder was obtained. This material was air-dried, transferred quantitatively to Soxhlet thimbles, and extracted twenty-four hours with redistilled, dry chloroform. The chloroform was removed and the residue dried in vacuo. The residual lipids were taken up in low-boiling petroleum ether and filtered through fat-free paper into a 100 ml. volumetric flask. The contents were then made to volume with ether and aliquots taken for gravimetric determination of total lipids. Additional aliquots were taken for determination of total and free cholesterol by the Schoenheimer-Sperry method[16]; of phospholipids and free fatty acids by the titrimetic method of Man and Gildea[17]; and for determination of total lipid phosphorous using the method of Youngberg.[18] The per cent of total lipids present as neutral fat was then calculated by difference. These data are summarized in Table VI. It is seen that the total lipid content of the six organs examined from each animal was not significantly different in the four infused animals or in the noninfused animals. The values obtained are similar to those reported by other workers[19] for normal dogs and confirm the histologic evidence which revealed no abnormal lipid deposits in the tissues.

The livers of the four infused animals showed an increase of total cholesterol over the controls. This increase is associated with an increase of the ratio between the free to total cholesterol components. This was the only tissue which showed an abnormality of cholesterol content. The changes were of the type already described in the plasma; the total cholesterol content is increased with a marked increase in the ratio of free to ester cholesterol. As noted, the plasma cholesterol ratios had returned to normal sixteen days after the last infusion.

DISCUSSION

The data obtained in these studies give additional support to findings from this laboratory that a properly prepared emulsion of triglycerides given intravenously is utilized for energy requirements. The use of litters of growing puppies has proved to be a sensitive indicator of these functions. It has also been shown that proper selection of phosphatide stabilizers and careful preparation will give a stable emulsion of fat which can readily be infused in amounts up to 5 Gm. of fat per kilogram of body weight per day. This amount of fat represents 20 to 30 per cent of the caloric requirement of an actively growing animal. Microscopic and chemical examination of the tissues of infused animals revealed minimal cellular reaction in the livers which is transient and after a few weeks leaves no apparent anatomical damage.

Although two of the puppies in Experiment 1 showed minimal evidence of histologic changes which may be attributed to the emulsions infused when sacrificed two to three weeks after the termination of the infusions, these changes were of questionable significance. Certainly there was no evidence of irreversible tissue damage as the result of continued infusions of large amounts of emulsion.

Animal 906 was found to be free of lesions four to five months later, and Dog 909 which had received over 3 kg. of fat intravenously in a period of sixty-three days showed no histologic evidence of damage when examined at the end of the infusion period. Furthermore, histologic data corroborate metabolic data indicating that the infused fat was disposed of in an orderly fashion and was apparently converted to energy and growth requirements. Even when as much as 10 Gm. of fat per kilogram per day was infused there was no evidence that fat was deposited in the liver or other organs to the detriment of function at these sites. Chemical analysis revealed a disturbance of cholesterol metabolism leading to an increase of free cholesterol with a concomitant reduction of ester cholesterol in the plasma. A similar change of the cholesterol distribution and content was found in the livers of all infused animals sixteen days after the last infusion. There was no evidence of excessive deposition of lipids in the liver or other visceral organs. Liver function tests did not indicate injury to this organ other than the disturbance of cholesterol metabolism. A moderate anemia was consistently observed in the infused animals but it seemed to be self-limited and improvement was prompt with cessation of the infusions. Since the anemia was of equal degree in the animals receiving only the phosphatide stabilizer, it appeared not to be due directly to the fat. In many respects this anemia resembled the anemia of infection.

The relationship of appetite to the amount of infused fat suggests that the oral intake is determined in part by the total caloric requirement. The parenteral use of fat emulsions of high caloric concentration allows a promising approach to the problem of supplying adequate calories in a small fluid volume for individuals who are in caloric deficit and unable to take sufficient calories by mouth. However, if calories provided by vein are sufficient to reduce markedly the appetite, and hence oral intake, one may soon encounter or aggravate complications related to an inadequate intake of protein, vitamins, or minerals unless care is taken to prevent the onset of such deficiencies.

The consistent observations that the immediate symptoms, the anemia, the transient liver lesions, and the disturbances of cholesterol metabolism were all as well marked in the animals receiving the phosphatide stabilizer alone as in the animals infused with fat emulsions suggest that despite efforts to improve and purify the phosphatide stabilizer this component of the emulsion was principally responsible for any undesirable effects that were produced.

SUMMARY

1. Two litters of puppies were used to assay the contribution of an intravenous emulsion of 30 per cent coconut oil to energy requirements.

2. As judged by growth and nitrogen balance, growing puppies were able to utilize up to 30 per cent of their total energy requirements supplied as fat emulsion intravenously.

3. Chemical data indicated no disturbance of plasma proteins, nonprotein nitrogen, bilirubin, or liver function during the infusions.

4. A moderate normocytic anemia developed in the animals that were infused, regardless of whether they were infused with fat emulsion or just

the phosphatide stabilizer. The anemia was self-limited, responded favorably when the infusions were stopped, and in many respects resembled the anemia of infection.

5. Histologic examination of the various visceral organs revealed no abnormal lipid retention. Occasional transient, focal collections of mononuclear cells were seen in the liver for a few weeks after the infusions.

6. Chemical lipid analysis revealed no lipid retention but did indicate a disturbance of cholesterol metabolism, both in the plasma during infusion and in the livers of infused animals.

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THE ELECTROPHORETIC ANALYSIS OF SERUM PROTEINS OF THE BLOOD DYSCRASIAS

RAY K. BROWN, M.D.,* JOHN T. READ, M.D., BRUCE K. WISEMAN, M.D., AND WESLEY G. FRANCE, PH.D.
COLUMBUS, OHIO

REPORTS of the electrophoretic analysis of serum proteins in the several diseases involving the blood cells or their precursors are uncommon in the medical literature. In most instances the data presented have been recorded incidental to broader considerations of protein analysis or secondary to hematologic and histopathologic studies. Numerous reports have been made of the serum protein changes in multiple myeloma and characteristic patterns have been recognized.[1, 2, 3, 4] However, none of these have considered the effect on the serum proteins of a recently suggested form of chemotherapy.[5] In view of the close relationship of diseases of the reticulo-endothelial system[6] and the probable sites of formation and alteration of the serum proteins, further investigation of the serum protein architecture of these related states appears indicated. In the present study the changes occurring by electrophoretic analysis of the serum proteins in various types of leucemia, pernicious anemia, infectious mononucleosis, polycythemia vera, and reticulum cell sarcoma are analyzed.

The object of this study was to learn (1) whether there is a pathognomonic serum protein architecture in one or more of the disease states under consideration and (2) if there is any correlation between the qualitative and quantitative changes observed, the related histopathology and the simpler clinical laboratory procedures.

MATERIALS

The patients used for this study were admitted to the University Hospital between Sept. 1, 1946, and Dec. 30, 1947. The diagnosis was established by history, physical examination, and blood and bone marrow studies using the supravital and standard Wright's staining techniques. Routine and indicated special laboratory procedures, including lymph node biopsy, were accomplished in each instance.

The distribution of patients into diagnostic categories was as follows: chronic myelocytic leucemia, three; acute myelocytic leucemia, two; chronic monocytic leucemia, two; acute monocytic leucemia, four; chronic lymphocytic leucemia, two; acute lymphocytic leucemia, one; multiple myeloma, four; reticulum cell sarcoma, one; polycythemia vera, one; infectious mononucleosis, two; pernicious anemia in relapse, two. The classification of patients and the acuteness or chronicity of a given case were determined by the cellular morphology of the peripheral blood and bone marrow in addition to the duration of illness prior to hospitalization.

METHODS

All the serum analyzed was collected under sterile precautions while the patients were in the fasting state, prior to transfusion and all but symptomatic therapy. The exception to this rule was the second sample taken from patients with myeloma who had been treated

From the Departments of Medicine and Chemistry, The Ohio State University, and the Starling-Loving University Hospital.
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*Electrophoretic analyses.

TABLE I

DISEASE	MOBILITY					ABSOLUTE CONCENTRATION						RELATIVE CONCENTRATION				
	ALB.	α_1	α_2	β	γ	ALB.	α_1	α_2	β	γ	T.P.	α_1/A	α_2/A	β/A	γ/A	A/G
Normal	6.86	4.30		3.00	1.06	3.89	.34		.98	.49	5.70	.088		.25	.12	2.17
Normal[10]	6.30	5.20	3.93	2.82	1.10	5.69	.28	.30	.82	.97	8.06	.049	.16	.14	.17	2.40
Normal[8]	5.94	5.07	4.08	2.83	1.02	4.04	.31	.48	.81	.74	6.38	.076	.12	.20	.18	1.72
Range +												.090–.150		.16–.26	.13–.25	1.59–2.39
1, a. Chronic myelocytic	6.24	5.11	3.83	2.82	.722	3.34	.52	.64	1.10	0.72	6.32	.16	.19	.33	.22	1.12
b.	6.38	5.24	4.03	3.08	.989	3.49	.36	.42	0.86	1.03	6.16	.10	.12	.24	.29	1.30
c.	6.22	5.09	4.12	2.99	.974	3.24	.58	.64	1.04	1.01	6.50	.18	.20	.32	.32	1.00
2, a. Acute myelocytic	6.18	5.12	4.07	2.94	.956	2.12	.56	.61	.98	.96	5.23	.21	.29	.46	.45	.68
b.	6.38	5.14	4.13	2.96	.957	2.79	.47	.59	.38	0.64	4.87	.17	.21	.14	.23	1.35
3, a. Chronic monocytic	6.32	5.29	4.31	3.12	1.03	4.88		.68	1.36	2.58	9.50		.14	.28	.52	1.06
b.	5.93	4.82	3.73	2.67	.835	4.18	.77	1.00	1.06	0.62	7.64	.18	.25	.27	.15	1.28
4, a. Acute monocytic	6.30	5.09	3.89	2.91	1.33	1.88	.51	.84	0.64	1.01	4.89	.27	.45	.34	.54	.627
b.	6.03	5.05	3.93	2.73	.95	1.88	.62	.71	0.92	0.70	4.87	.33	.38	.46	.37	.640
c.	6.52	5.47	4.50	3.25	1.36	5.12	.49	.57	1.13	1.58	8.88	.095	.11	.22	.31	1.37
d.						3.77	.93	1.31	1.54	1.25	8.79	.25	.35	.48	.33	.745
5, a. Chronic lymphocytic	6.20	5.08	4.01	2.93	.967	2.58	.42	.57	0.50	.28	4.35	.16	.22	.20	.11	1.54
b.	6.26	5.22	4.12	2.96	.895	2.26	.48	.62	0.49	0.33	4.18	.21	.27	.23	.15	1.14
6, a. Acute lymphocytic	6.22	5.07	3.96	2.93	.96	2.02	.46	.53	.61	.73	4.35	.23	.21	.25	.36	.867
7. Reticulum cell sarcoma	6.28	5.02	4.06	2.97	.786	2.06	.35	.41	.57	2.20	5.59	.17	.20	.28	1.0	.60
8. Polycythemia rubra vera	6.09	5.08	3.09	2.89	.890	2.25	.33	.51	.25	.29	3.62	.15	.22	.30	.14	1.63
9, a. Infectious mononucleosis	5.81	4.75	3.92	2.76	.99	3.29	.37	.50	.98	1.41	6.55	.114	.15	.30	.43	1.00
b.	6.33	5.20	4.00	2.89	.97	3.76	.36	.48	.98	1.51	7.08	.096	.13	.26	.41	1.13
10, a. Pernicious anemia-relapse	6.42	5.36	4.08	2.82	.92	4.68	.46	.49	.98	.87	7.46	.10		.21	.12	1.65
b.	6.02	4.97	3.97	2.98	.82	4.22	.61		.91	.74	6.47	.15–		.21	.17	1.87

+9, Range into which 68 per cent of normal subjects fall.

with Stilbamidine. After collection the serum was refrigerated at −20° C. and the analysis was made after an interval of three to twenty-one days had elapsed. Aseptic technique was employed up to the time the serum was dialyzed preparatory to electrophoresis.

The frozen samples were thawed and diluted from 7 volumes to 25 volumes with 0.10 ionic strength barbiturate buffer at pH 8.6. The diluted serum was then dialyzed at a temperature of 1° C. against eight to ten times its volume of buffer, thrice changed during a 72- to 168-hour period. The dialyzed samples were then examined in a standard Klett electrophoresis apparatus using Longsworth's technique.[7] A 2 c.c. cell was employed for intervals up to 220 minutes at potential gradients of about 5 volts per centimeter at thermostat temperatures of about 1.0° C. Several photographic exposures of each run were made. The conductivity readings for the descending mobilities were made on the sample, while those for the ascending mobilities were made on the buffer at 0° C.

Mobilities were determined from three different plates and averaged for each ascending and each descending boundary. These two figures were then averaged to give the figure reported. Concentrations were estimated by making enlargements from the photographic plates so that the new areas were from five to forty times the areas on the plates. The resulting areas were then traced with a planimeter four times and the results averaged. This procedure was done once for each ascending and descending component. The values were then converted to per cent protein using $\Delta n = .00200$/per cent, for albumin, and $\Delta n = .00219$/per cent for all globulins. Unless otherwise indicated the results given are the average of the values from the ascending and descending boundaries.

RESULTS

The data from the electrophoretic patterns of the sera of the various disease entities are set forth in Table I. Two of the normal analyses determined by us are added as well as the normal limits of mobilities, approximate relative and absolute concentrations of the various fractions.[8]

In the group of chronic leucemias listed, there is a qualitative deviation from normal in the gamma globulin boundary which migrated at a slower rate than would be anticipated normally. This phenomenon was observed in all types of chronic leucemia and was present in the sera from the patient with polycythemia vera and the one with reticulum cell sarcoma. Generally the leucemic sera showed a decrease in the approximate absolute concentration of albumin. The two cases of chronic monocytic leucemia and one of the acute cases had normal albumin values. The decrease in albumin in leucemia was suggested by Keilhack[9] using the Howe technique of sodium sulfate precipitation. One of the patients with chronic monocytic leucemia (3a) was seen early in the disease and at that time had a high normal albumin concentration. As the disease progressed this value became markedly lower.

The leucemic sera rather uniformly demonstrated a rise in the approximate absolute and relative concentration of alpha-1 and alpha-2 globulin. The cases of reticulum cell sarcoma and polycythemia were slightly elevated in this moiety. These globulins were within normal limits in the sera of the cases of pernicious anemia and infectious mononucleosis.

The leucemic sera in general (except the chronic lymphatic) demonstrated a rise of the approximate absolute and relative concentration of beta and gamma globulin. The sera of the chronic lymphocytic leucemias were below normal for gamma globulin. Both cases of infectious mononucleosis demonstrated a rise in the relative and absolute values for gamma globulin. Sera from the patient

with reticulum cell sarcoma showed a marked rise of gamma globulin both relative and absolute, and normal beta globulin. The case of polycythemia presented low values for beta and gamma globulin. The early case of chronic monocytic leucemia and the acute monocytic leucemias demonstrate a rather marked increase in the relative gamma globulins. The cases of pernicious anemia were within normal limits for the approximate relative and absolute values of beta and gamma globulin. The total protein concentration values of all sera

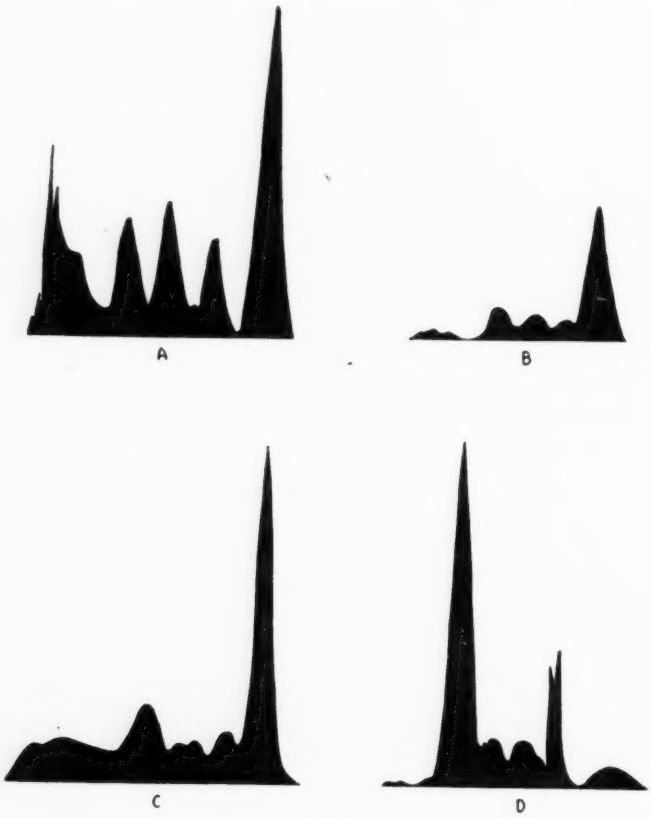

Fig. 1.

fractionated were not comparable with those determined by the routine clinical methods, and in all but one sample the albumin-globulin ratio was lower when computed on the basis of the electrophoretic analysis. These albumin-globulin ratios determined by electrophoresis fall well below the accepted normal values.[10]

Representative schlieren patterns are shown in Fig. 1. Sera from one of the patients with acute monocytic leucemia demonstrated a small spike on the gamma globulin peak which we have not observed in normal subjects and which was seen in previous analyses of this type of sera. (Fig. 1, *A*.) Fig. 1, *B* is a pattern from a patient with chronic lymphocytic leucemia which displays a very low gamma globulin peak. One pattern (Fig. 1, *C*) from a case of monocytic leucemia shows a notching of the alpha-2 peak which was present but not

TABLE II

CASE	MOBILITY					ABSOLUTE CONCENTRATION						RELATIVE CONCENTRATION					STIL-BAMIDINE	MARROW
	ALB.	α_1	α_2	β	γ	ALB.	α_1	α_2	β	γ	T.P.	α/A	α/A	β/A	γ/A	A/G		
1	5.99	5.00	3.85	2.82	1.04	1.47	.26	.32	5.61*	.22	8.10	.17	.22	3.13*	.16	.27*		48% Plasma cells
2, A	6.29		3.88	2.82	.88	3.68	.50	.98	1.08†	7.50	13.24	.14	.26	.32	2.04	.39	2,250 mg.	84% Plasma cells
B	6.16	5.20	4.04	2.92	.87	3.61	.60	.69	1.20	10.07	15.00	.17	.20	.35	3.22†	.24		71% Plasma cells
3, A	6.00	5.00	3.68	2.13	.90	2.31	.36	.87	6.63*	.12		.17†	.31†		.58		3,300 mg.	57% Plasma cells
B	5.88	4.97	3.84	2.62	1.18	3.40	.31	.38	12.74	1.34	18.19	.09	.11		.37	.23		55% Plasma cells
4, A	6.33	5.47	3.94	3.17	.77	4.79	.56	.16	.89†	14.52	20.92	.12	.34	.19	3.03	.29	3,300 mg.	40% Plasma cells
B	5.64	4.67	3.60	2.58	1.16	4.26	.32	.54	1.00	11.43*	17.27*	.09	.12	.24	2.16*	.39*		36% Plasma cells

*Descending only.
†Ascending only.
A, Before treatment.
B, After treatment.

as marked in chronic myelocytic leucemia. Fig. 1, *D* is from a case of chronic myelocytic leucemia and discloses an unusual abnormality of the beta globulin spike.

The data from sera of patients having a confirmed diagnosis of multiple myeloma are presented in Table II. The architecture before treatment and in the untreated cases confirms the report by Kekwich[1] that there are two types of abnormal patterns in myeloma, a high beta globulin peak and a high gamma globulin peak. There seems to be no obvious correlation that can be made regarding any quantitative or qualitative changes in the peripheral blood protein pattern after the administration of Stilbamidine. The values are shown in Table II.

DISCUSSION

In a study of the changes in mobility of the electrophoretic pattern of serum following experimental burns, Perlmann and co-workers[11] found a new boundary which migrated at a slower rate than gamma globulin. Seibert[12] noted a similar component in half of the tuberculosis cases analyzed. One analysis of a patient with acute lymphocytic leucemia reported by Longsworth and associates[13] shows a tendency to slow mobility at the gamma globulin boundary. Though our cases of chronic leucemias showed this tendency to slowing, none of them displayed a separation of the peak into two parts.

The generally lowered absolute concentration of albumin has been noted in various pathologic states. Leutscher,[14] in his review of the subject of electrophoresis, points out the common occurrence of this trend and hence it cannot be considered as characteristic of the blood dyscrasias. This deviation from normal has been pointed out by other authors [9, 13] in both acute lymphocytic and myelocytic leucemias.

The absolute concentrations of the alpha-1 and alpha-2 globulins were generally increased over normal in the leucemias of this study. It has been stated that an increase in the alpha globulin is the first change which takes place in acute injury or infection.[14] The fraction of alpha globulin which is responsible for this increase was not designated. However, it was present in the chronic as well as the acute leucemias of this series. The relative values of alpha-1 and alpha-2 globulin were similarly increased. Chow[16] has shown that the rise of alpha globulin may be dependent on a fall of albumin, with normal values for the alpha globulin attained by increased protein intake and restitution of the albumin fraction. However, in several of our cases (Table I, 3,b, 4,c) there were elevated absolute alpha globulins, the albumin fraction being within normal limits. Furthermore, in one case of chronic lymphocytic leucemia the administration of 50 Gm. of serum albumin daily for a period of fourteen days restored the albumin to normal levels and only slightly altered the alpha-1 globulin. However, the alpha-2 globulin approximated normal and the gamma globulin content remained essentially the same. In one case of chronic myelocytic leucemia, placing the patient on a high protein diet supplemented with protein hydrolysates did not restore the albumin fraction or alter the alpha globulins appreciably even though all liver function studies were within normal limits.

A relative elevation of the gamma globulin is said to be a phenomena observed in the chronic state of disease and injury.[14] Our data would tend to support this, though the aberration was prominent in some acute cases and absent in a few of the chronic ones.

Both cases of chronic lymphocytic leucemia present a low value for both relative and absolute gamma globulin. Howell[17] noted that leucemic individuals do not respond normally to infection or to injected antigens. Krebs[18] noted diminished value of gamma globulin in simple malnutrition. One patient with chronic lymphocytic leucemia given a series of injections intravenously of typhoid vaccine did not develop antibodies as shown by the Widal test which remained negative. The administration of extract of the adrenal cortex did not alter the antibody response or the electrophoretic pattern. Repetition of this experiment after restitution of the albumin fraction did not influence the gamma globulin. Blood volume determinations[19] prior to and following the administration of extract of the adrenal cortex demonstrated a moderate increase and this factor was taken into account in the latter determination. It is possible however that the pituitary adrenotrophic hormone may be involved in the gamma globulin component as Dougherty and White have pointed out in individuals with normal lymphocytes.[20]

In the cases of monocytic leucemia and reticulum cell sarcoma, marked elevation of absolute and relative gamma globulins is seen. The explanation for this rise is obscure but may be related to the similarity of the fundamental histopathologic defect in both entities, the reticulum cell and the monoblast being closely related in the polyphyletic tree.[21]

Both patients with infectious mononucleosis had elevated absolute and relative gamma globulin values and moderate diminution of the albumin fraction. The heterophile antibody was strongly positive in both cases. Although the etiology of this disease is unknown,[22] much evidence has been submitted[23] demonstrating the widespread involvement of the reticulo-endothelial system and other organ systems which could account for the deviation of the protein pattern from normal. The cases of pernicious anemia in relapse both demonstrated normal albumin values and normal globulin fractions. As would be expected from the approximate relative or absolute increase of the various globulin fractions, the albumin-globulin ratio was consistently decreased, often to subnormal levels and even less than 1.0. All were below the range within which 68 per cent of the normal cases should fall, and most were not within the limits which includes 94.5 per cent of normal subjects.[9]

In Table III there is a comparison of the electrophoretic protein determinations and the results of the clinical laboratory procedures performed in the respective disease entities. The routine laboratory protein determinations were not comparable to the total protein or A/G ratios as determined electrophoretically. The latter technique in most instances gave uniformly lower ratios than those obtained by the routine clinical laboratory methods. Nitske and Cohen[24] recently have demonstrated the inadequacy of the routine total protein determinations in cases of Hodgkin's disease and leucemia. These authors, utilizing a methyl alcohol fractionation technique, demonstrated lower albumin and elevated globulins by this method than by the usual clinical methods.

TABLE III

DISEASE	TOTAL PROTEIN	A/G	ELECTROPHORETIC TOTAL PROTEIN	A/G	B.U.N.	B.M.R.	THYMOL	CEPH. FLOC.	PROTHROMBIN	ACID PHOSPHOTASE	W.B.C.	ABSOLUTE IMMATURE	MARROW
Chronic myelocytic	6.66	2.74	6.32	1.12	21.5	+49	20.0	+	30.5	5.4	742,000	497,000	Myeloid hyperplasia "B level." Blast cells 6%
	6.94	1.81	6.16	1.30	11.5	+22	0.0	−	42.0	4.68	206,000	127,000	Myeloid hyperplasia Mature level Blasts 2%
	6.35	2.68	6.50	1.00	18.0	+46	0.0	+	59.0	6.4	358,000	207,000	Myeloid hyperplasia "C" level Blasts 3%
Acute myelocytic	5.71	1.65	5.23	.68	7.5	+36	20.0	+	54.0	7.6	48,000	26,400	Myeloid hyperplasia Blasts 55%
	5.94	1.45	4.87	1.35	12.0	+32	10.0	+	60.0	3.8	76,000	31,920	Myeloid hyperplasia Blasts 42%
Chronic monocytic	8.68	1.00	7.64	1.28	13.5					3.7	24,000	2,880	Monocytes 39% Monoblasts 6%
	8.52	.98	9.50	1.08	10.6	+18	10.0	−	114.0	3.5	1,350	68	Monocytes 31% Monoblasts 8%
Acute monocytic	6.66	1.51	4.89	.63	20.0		40.0	+	48.8		500	155	Monocytes 4% Monoblasts 31%
	5.94	1.45	4.87	.64	17.0	+36		+	53.8		1,550	62	Monocytes 5% Monoblasts 11%
	6.25	1.47	8.88	1.37	11.5		20.0				1,800	144	Monocytes 1% Monoblasts 17%
	7.48	1.22	8.79	.74	15.5		10.0	±			2,250	315	Monocytes 20% Monoblasts 18%

Chronic lymphocytic	5.78	4.78	4.18	1.14	14.0	+43		−		5.1	325,000	0	Lymphatic infiltration 95%
	5.74	2.42	4.35	1.54	16.2	+30		+	85.0	3.4	294,000	0	Lymphatic infiltration 75%
Acute lymphocytic	5.42	2.03	4.35	.87	33.8	+60	10.0	±	69.0	4.6	66,000	61,380	Lymphatic infiltration 90%
Reticulum cell sarcoma	7.06	1.00	5.59	.60	14.0	+38	40.0	++++	23.0	3.54	8,100	0	Sarcoma cells 32%
Polycythemia rubra vera			3.62	1.63	14.5	+20	0.0	++	−	4.20	92,000	16,560	Generalized hyperplasia, all elements
Infectious mononucleosis	6.98	1.74	6.55	1.0	13.2	−4	5.0	++	92.0	3.0	4,500	3,600*	Not done Heterophile 1:7168
	8.57	1.44	7.08	1.13	10.0	+8	10.0	+++	100.0	3.2	13,000	8,515*	Rieder cells 4% Heterophile 1:14336
Pernicious anemia relapse	4.74	2.40	6.47	1.87	24.0		10.0	±	52.0	4.4	10,900	0	Megaloblast-hyperplasia 32
	5.44	1.86	7.46	1.65	16.0		15.0	+	61.0	3.8	3,900	0	Megaloblast-hyperplasia 26

*Rieder cells.

The correlation of the changes in protein architecture and the varied clinical laboratory procedures are particularly difficult to evaluate as the primary disease process, and many secondary effects of the disease may alter each determination. The albumin-globulin ratio or total protein determination did not bear any consistent relationship to the other procedures. The basal metabolic rate and the uric acid determinations were more consistently involved or altered in the leucemic state. These two determinations have been repeatedly investigated in this condition and are assumed to be elevated by increased protein catabolism and the breakdown of leucocytes.[25] The cephalin flocculation and thymol turbidity tests were markedly elevated and the prothrombin time diminished when the excretory tests of liver function showed alteration. The thymol turbidity and cephalin flocculation tests in low titers bore no constant relationship to an abnormal A/G ratio and their aberrations may be explained in part by the occurrence of abnormal amounts of beta and gamma globulin in the sera. Cohen[26] has demonstrated by electrophoretic studies the relationship between the thymol turbidity test and the beta globulins. The cephalin flocculation test is also reported by this author to be more closely parallel to increases of gamma globulin.

The degree of infiltration of the bone marrow by leucemic cells was determined by performing four differential counts of 200 myeloid cells each and averaging the results. By this gross determination the degree of infiltration appeared to reflect itself in a lower A/G ratio by the electrophoretic technique. No correlation between the small numbers of plasma cells in the bone marrow, lymph nodes, or autopsy specimens and the protein architecture was noted.

Investigation of myeloma serum by electrophoresis has been as thorough as that of any single disease, and its schlieren patterns can be as nearly pathognomonic as those seen in any morbid state. As stated, our results confirm those in the literature which refer to two abnormal types of myeloma patterns, one with a marked increase in the area of the beta globulin (cases 1 and 3) and the other with the major protein increase in the gamma globulin fraction (cases 2 and 4). In treated patients, the one with the beta elevation prior to treatment showed an increase in this fraction following therapy. In the gamma type no consistent effect of Stilbamidine on the protein architecture was observed. No significant changes were noted in the peripheral blood or bone marrow that could be ascribed to the therapy.

SUMMARY

The electrophoretic patterns of various blood dyscrasias are presented. The leucemic states are associated with a diminution in the approximate absolute amount of albumin and a rise in the absolute amount of globulin. The albumin-globulin ratios fall below the limits of normal in most instances. The alpha-1 and alpha-2 globulin are increased in most instances and the increase is noted with both normal and diminished total albumin values. Gamma globulin values, both absolute and relative, were elevated in monocytic leucemia, reticulum cell sarcoma, and infectious mononucleosis. Chronic lymphocytic leucemia demonstrated low relative and absolute gamma globulin values. A markedly lowered albumin-globulin ratio appears related to the degree of infiltration of the bone

marrow by leucemic cells as well as when the excretory and metabolic functions of the liver demonstrate impairment. No alteration in the serum protein architecture was noted following Stilbamidine therapy.

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ELECTROLYTE PARTITION IN PATIENTS WITH EDEMA OF VARIOUS ORIGINS

Qualitative and Quantitative Definition of Cations and Anions in Cardiac Decompensation

Edith B. Farnsworth, M.D., and Jacob S. Krakusin, M.S.
Chicago, Ill.

With the Technical Assistance of Lora Belle Hughes, B.A., M.T. (ASCP)

THE role of the kidneys in the production of edema has been examined by various groups of investigators[1-5] as methods for the study of renal physiology have been developed. Although agreement has not been general concerning the sequence of events transpiring in the kidneys during the accumulation in the tissues of edema fluid, it is at least conceded that such events do transpire and that salt and water are not simply diffusing across the capillary membrane in response to an elevation of hydrostatic pressure, but are being incompletely excreted by the kidney. This concept is by no means novel, and appears in standard texts of a decade or two ago.[6] Further clarification, however, has been delayed by the difficulties, inherent in the study of renal physiology in man, of establishing values for filtration rate sufficiently accurate to serve as a basis for the calculation of reabsorbed substances. The volume of plasma worked by the kidneys in a given period of time is so large, and the defect in salt and water excretion capable of leading to edema is relatively so small, that the significant increment tends to be lost in the physiologic variations of the inulin or mannitol clearances as we are able to determine them at the bedside. Thus, if the clearance of inulin is found to vary only to the extent of plus or minus 5 or 10 c.c. per minute, that range of error, or physiologic variation, is so large in relation to the few cubic centimeters of water or fractions of milliequivalents of sodium which represent the retention characteristic of cardiac decompensation, that physiology described in these terms must be accepted with reserves. Even more inaccessible to the clinical investigator are determinations capable of yielding conclusive information concerning the factors governing diffusion and active reabsorption in proximal and distal tubules and the stimuli which may modify these processes in disease states.

Bearing in mind these limitations, we have chosen to review in detail the alterations in urine electrolytes earlier reported in group studies[5] confined to sodium and chloride. The depression of urinary sodium-chloride ratio in cardiac failure appeared to be a constant finding implying a higher degree of specific selective tubular reabsorption than has been generally recognized. Through a more complete delineation of relationships, with varying electrolyte

From the Departments of Medicine and Chemistry, Northwestern University Medical School.

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loads, it was our purpose to define the variation of electrolyte patterns and fluid balance with sufficient accuracy and completeness as to bring out characteristics tending toward a recognition of possible causal agents. In adopting an approach similar to that of Gamble[7] we were able to avail ourselves of accepted data pertaining to the normal state.

METHODS

The two subjects selected for this study were suffering from rheumatic heart disease with congestive failure. E. W. was a 36-year-old woman with mitral stenosis and insufficiency, who was hospitalized in congestive failure. She was discharged improved after the experimental period but was twice rehospitalized during the subsequent six months because of fluid retention.* W. S. was a 28-year-old man who was first seen in the clinic and hospitalized because of advanced rheumatic heart disease with congestive failure. He was discharged improved after the reported period of study and treatment but was later admitted to a veteran's hospital in terminal condition. The autopsy report indicated rheumatic involvement of the aortic and mitral valves, cardiac cirrhosis, and pulmonary infarction.

Both patients were kept at bed rest for the first fortnight of their hospital stay, after which they were permitted to move about the wards as they wished. Both were allowed fluids ad libitum and both were given a low-salt, acid ash diet. The estimated electrolyte content of the diet, in millimols per day, was:

Sodium	17-64 (average 44)
Potassium	103
Calcium	13
Magnesium	12
Iron	4
Phosphorus	63
Sulfur	32
Chloride	13-46 (average 30)

Urine specimens were collected under toluene and were kept refrigerated throughout the collection period. pH was determined on the samples as soon as they were received in the laboratory. If ammonia and phosphate determinations could not be carried out immediately, aliquot samples were frozen in solid carbon dioxide and were maintained at -70° in a Dewar flask until ready for use.

Analytic procedures used in the determination of ionic constituents were for the most part as recommended by Peters and Van Slyke.[8] All determinations were performed in duplicate or triplicate.

Sodium.—Gravimetric method of Butler and Tuthill. Some of the gravimetric urinary sodium values were checked, using a colorimetric adaptation of this procedure.

Potassium.—Gravimetric procedure of Freeman and Burrill.[9]

Calcium.—Microtitration by the method of Tisdall and Kramer.

Magnesium.—Precipitation according to the method of Simonsen, Westover, and Wertman.[10] Color development, however, was by the phospho-molybdate technique of Gomori.[11]

Ammonia.—Determination was by nesslerization following aeration from alkaline solution as in the method of Folin and Farmer.[12]

Chloride.—Modified Volhard-Harvey titration. In many instances, especially when protein was present, these were checked by the Van Slyke-Hiller[13] modification of Sendroy's iodometric procedure.

Phosphate.—The method of Gomori[11] was used after decolorization and dilution. Milligrams phosphorus were converted to milliequivalents phosphate ($HPO_4^=$) by use of the appropriate factor as indicated by the pH of the sample.

Inorganic Sulfate.—Turbidimetric measurement of the barium sulfate precipitate.[14] This is admittedly a comparatively crude method but it gave satisfactory results and recoveries.

*Recently reported deceased at home.

TABLE I. URINE ELECTROLYTES (IN MEQ./24 HOURS); PATIENT E. W.

DATE 1947	INTAKE	R	OUTPUT	pH	Na^+	K^+	NH_4^+	Ca^{++}	Cl^-	$HPO_4^=$	INORGANIC $SO_4^=$	ORGANIC ACIDS	WT.
April 13	(Admitted to hospital)												143¼
April 15	1500	No medication	800	6.6	7	16	6	11.0	20	15	11	14	144¾
April 16	2000	No medication	1020	6.4	4	33	17	13.6	12	20	16	21	145¼
April 17	1650	No medication	1168	6.9	2	17	15	14.5	8	16	10	16	145¾
April 18	1200	No medication	1180	6.7	2	37	20	11.3	13	20	14	22	146¼
April 19	1450	8 Gm. NH_4Cl	1500	6.8	5	54	13	15.0	23	23	16	30	148½
April 20	1250	8 Gm. NH_4Cl	910	6.5	2	66	16	10.1	48	16	11	24	147
April 21	1800	12 Gm. NH_4Cl	1265	6.3	1	50	43	16.9	82	24	18	31	146½
April 22	1900	12 Gm. NH_4Cl	1320	6.4	2	131	38	18.1	103	16	12	41	147
April 23	1700	12 Gm. NH_4Cl	1380	6.1	22	61	47	17.5	99	21	15	26	147½
April 24		12 Gm. NH_4Cl	2110	6.1	7	68	53	13.1	92	20	11	23	147¼
April 25	1600	12 Gm. $CaCl_2$	1920	5.6	35	104	63	33.2	177	21	17	39	148
April 26	1350	12 Gm. $CaCl_2$	1720	5.6	32	57	40	34.7	134	6	8	17	146½
April 27	1700	12 Gm. $CaCl_2$	1550	5.8	3	63	53	48.2	107	16	13	21	145
April 28	1550	12 Gm. $CaCl_2$	1105	5.8	2	29	28	37.8	102	15	15	18	146
April 29	1930	12 Gm. $CaCl_2$	1115	5.9	5	34	29	38.8	95	15	13	24	146½
April 30	1600	12 Gm. $CaCl_2$	1690	5.9	6	43	32	33.3	77	12	11	22	147
May 1		12 Gm. $CaCl_2$	1195	5.6	6	56	33	23.8	48	18	18	19	147½
May 2	2100	12 Gm. NH_4NO_3	1005	5.8	2	16	63	13.0	39	15	13	20	
May 3	1350	12 Gm. NH_4NO_3	1155	6.2	2	22	61	12.8	23	12	13	20	147
May 4	1850	12 Gm. NH_4NO_3	1185	6.2	2	24	49	13.8	25	11	18	19	146½
May 5	1600	12 Gm. NH_4NO_3	990	6.2	2	24	73	11.4	21	8	66	16	147
May 6	1950	12 Gm. NH_4NO_3	1250	6.0	2	28	31	10.3	21	14	13	14	147½
May 7	1650	12 Gm. NH_4NO_3	980	5.8	1	31	31	10.1	20	11	11	15	147¾
May 8	1550	12 Gm. NH_4NO_3	1310	5.9	2	41	38	17.5	41	17	20	30	148¾
May 9	1300	12 Gm. NH_4NO_3	1050	5.9	1	24	33	7.4	28	13	11	15	148
May 10	1500	Mercupurin 2 ml.	2410	6.3	126	50	30	16.2	179	16	12	23	148¼
May 11	1900	No medication	1265	5.9	2	24	45	10.8	29	16	14	22	146½
May 12													146¾

Organic Acids.—Total twenty-four hour excretion was calculated from aliquots titrated according to Van Slyke and Palmer. Creatine and creatinine were removed by shaking with Lloyd's reagent as recommended by Greenwald.[15] Titration was carried out between pH 8.0 and 2.7, using a glass electrode for determination of end points. Correction for the existence of some of the acid in the free form as recommended by Gamble[7] was not made because titration curves indicated such a multiplicity of constituents that it was impossible to estimate which was the predominant acid.

Filtration rates and effective renal plasma flow were determined by the clearances of mannitol and sodium para-amino-hippurate. Analytic procedures were those of Corcoran and Page[16] and H. W. Smith and associates.[17]

Serum electrolyte determinations were made by adaptations of the methods already described for urine.

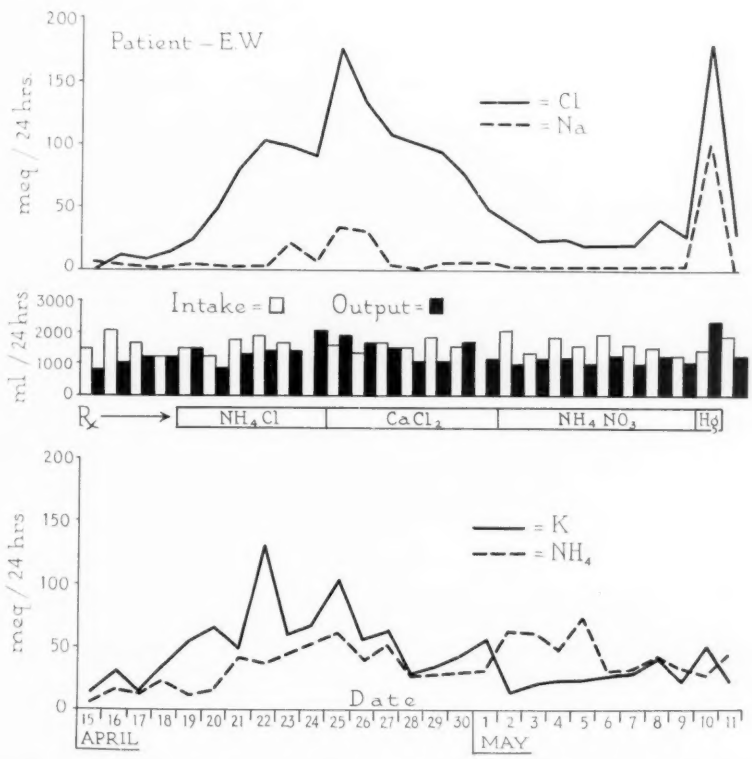

Fig. 1.—E. W.: electrolyte and water excretion with diuretics.

RESULTS

During control periods as throughout the administration of diuretics, sodium was consistently low both in relation to intake, total ionic concentration, and to chloride.

Other electrolytes showed no significant variation. E. W. maintained a consistently higher level of calcium output than did W. S., a finding consistent with previous observations that individual levels tend to be constant though variable from subject to subject.[18]

Of the mineral diuretics administered, ammonium chloride, potassium chloride, and potassium acetate appeared to be most effective as diuretic agents. It is notable that the maximum effect of each of these substances was reached around the third day of therapy, after which both fluid and electrolyte values declined toward control levels. For example (Fig. 2), on May 23 the urine volume had risen from 1,590 to 2,230 c.c. with an almost identical intake, and the sodium and chloride, respectively, from 5 to 75 meq. and 34 to 160 meq. After this diuretic peak, the output of salt and water declined notwithstanding the continuation of the same medication.

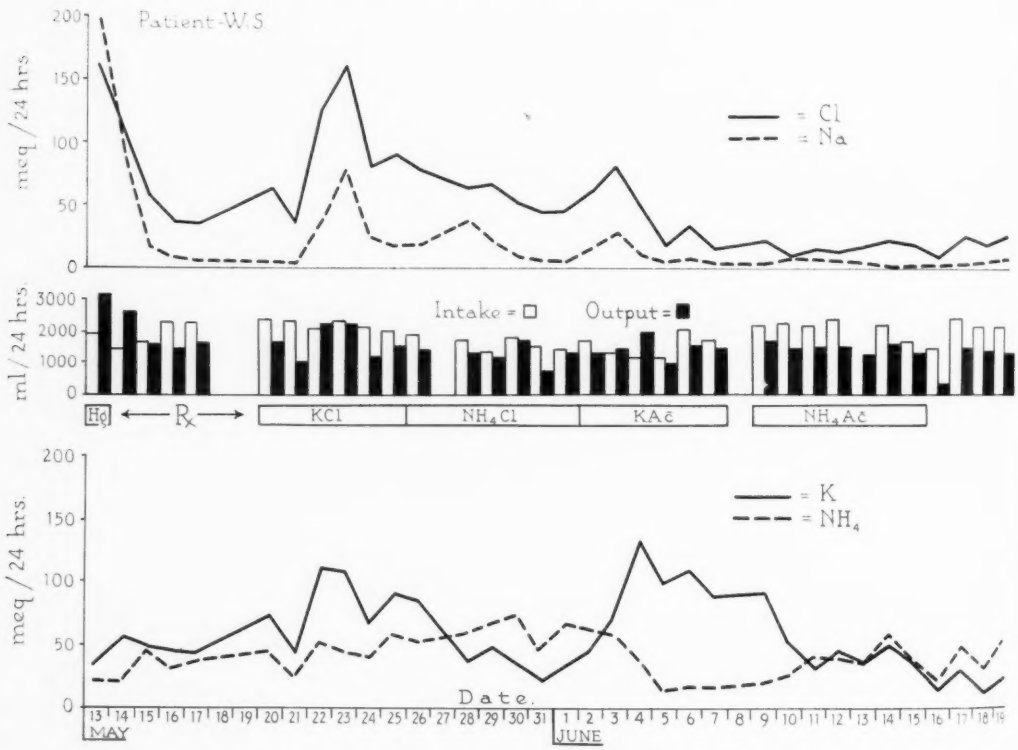

Fig. 2.—W. S.: electrolyte and water excretion with diuretics.

Although minor responses in the sodium output were found with ammonium chloride, potassium chloride, and potassium acetate (Figs. 1 and 2), the daily output tended to remain at a level far below normal values on varying salt intakes.[5, 19]

Certain findings peculiar to the specific diuretics are notable. Calcium excretion is distinctly increased with ammonium chloride; thus W. S. (Table II) on May 30 showed a calcium value of 11.3 meq., with 5.9 meq. before the beginning of the drug. Potassium acetate was effective in increasing water output, but the urine resulting from its administration was poor in sodium and chloride. As previously reported,[20] a prompt and sustained alkalinizing effect was demonstrated.

TABLE II. URINE ELECTROLYTES (IN MEQ./24 HOURS); PATIENT W. S.

DATE 1947	INTAKE	Rx	OUTPUT	pH	Na^+	K^+	NH_4^+	Ca^{++}	Mg^{++}	Cl^-	$HPO_4^=$	INORGANIC $SO_4^=$	ORGANIC ACIDS	WT.
May 10	(Admitted to hospital)													127½
May 11		Mercupurin 2 ml.												122½
May 13	1850	Mercupurin 2 ml.	3225	7.0	197	37	22	4.4	6.1	159	59	11	30	118
May 14	1400	No medication	2600	6.2	90	56	21	3.3	6.6	106	39	15	40	115
May 15	1700	No medication	1570	5.5	16	48	44	2.0	3.8	58	24	17	25	
May 16	2300	No medication	1400	5.6	9	45	32	2.4	3.4	36	17	16	22	114
May 17	2250	No medication	1590	5.6	5	44	36	2.4	3.7	34	20	19	24	114
May 20	2350	8 Gm. KCl	1650	6.0	4	74	45	5.8	6.3	63	26	25	25	115
May 21	2300	8 Gm. KCl	1010	5.6	3	44	24	2.8	3.1	34	12	13	22	115
May 22	2100	8 Gm. KCl	2220	5.8	35	111	50	5.2	5.0	124	24	25	29	115¾
May 23	2330	8 Gm. KCl	2230	5.6	75	108	45	7.0	3.7	160	24	30	35	115¾
May 24	2100	8 Gm. KCl	1150	5.2	23	68	40	3.4	2.7	80	18	16	17	115½
May 25	1990	8 Gm. KCl	1520	5.2	18	89	57	5.9	3.1	89	23	25	24	115½
May 26	1900	12 Gm. NH_4Cl	1370	5.4	17	84	53	5.7	2.5	78	22	26	27	116
May 28	1700	12 Gm. NH_4Cl	1240	5.1	35	38	58	10.3	3.9	63	20	23	25	116¼
May 29	1300	12 Gm. NH_4Cl	1060	5.0	20	48	67	10.6	4.5	66	19	17	24	115
May 30	1800	12 Gm. NH_4Cl	1680	5.4	9	34	72	11.3	4.0	52	19	27	27	–
May 31	1550	12 Gm. NH_4Cl	665	5.4	5	23	47	8.0	2.6	43	15	14	20	–
June 1	1400	12 Gm. NH_4Cl	1290	5.5	4		66	7.9	3.5	46	15	21	21	117
June 2	1650	10 Gm. potassium acetate	1240	5.3	15	45	62	8.7	2.2	60	15	22	27	115½
June 3	1300	10 Gm. potassium acetate	1370	5.3	26	71	56	6.4	3.5	82	16	25	27	116¾
June 4	1200	10 Gm. potassium acetate	1940	7.0	9	132	39	4.1	2.2	46	29	34	32	116
June 5	1130	10 Gm. potassium acetate	890	8.0	5	99	14	2.5	2.5	16	27	19	22	116½
June 6	2100	10 Gm. potassium acetate	1530	7.1	6	109	17	2.9		31	29	27	30	117½
June 7	1700	10 Gm. potassium acetate	1410	7.3	3	90	16	3.1	2.4	15	27	18	24	118
June 9	2180	8 Gm. ammonium acetate	1640	6.9	3	92	19	3.2	2.8	21	29	21	24	118¼
June 10	2250	8 Gm. ammonium acetate	1420	5.9	7	52	25	3.5	3.5	10	21	18	26	118½
June 11	2200	8 Gm. ammonium acetate	1440	5.5	7	34	40	4.7	2.9	14	22	21	25	118¼
June 12	2400	8 Gm. ammonium acetate	1480	5.5	5	45	39	6.7	2.7	13	23	24	32	118
June 13		8 Gm. ammonium acetate	1240	5.7	4	37	35	5.3	3.5	17	23	17	28	118
June 14	2200	8 Gm. ammonium acetate	1610	5.8	3	49	58	6.8	3.3	22	21	22	35	119¼
June 15	1740	8 Gm. ammonium acetate	1320	5.6	3	37	39	5.4	1.5	18	17	17	23	118½
June 16	1400	No medication	310	5.7	1	15	23	2.4	1.1	8	9	10	13	118½
June 17	2450	No medication	1440	5.7	3	32	48	5.1	2.5	24	23	25	26	119½
June 18	2100	No medication	1360	5.6	4	14	33	5.7	1.9	19	13	14	25	119½
June 19	2150	No medication	1240	5.5	7	23	53	4.5	1.8	24	14	17	26	120

Mercurial diuresis in W. S. resulted in a sodium value of 197 meq., with a chloride of 159 meq., giving a ratio of 1.2. In the case of E. W., the increase in sodium excretion was substantial but not sufficient to approach a normal ratio with the chloride. In one instance (W. S.) the discrepancy between sodium and chloride appeared to be compensated by a threefold increase in phosphate excretion. Other electrolytes were unaffected.

Filtration rates and effective renal plasma flow are given in Table III. The filtration rate in the case of E. W. was within normal limits before and throughout the period of this study. That of W. S., was reduced to approximately 50 per cent of normal. This marked disparity in filtering power was accepted as a desirable qualification in the selection of these subjects.

TABLE III

PATIENT	FILTRATION RATE (ML./MIN.)	EFFECTIVE RENAL PLASMA FLOW (ML./MIN.)
E. W.	119*	534*
W. S.	56†	303†

*Average of twelve determinations.
†Average of three determinations.

DISCUSSION

The choice of total quantitation of individual electrolytes as an approach to the evaluation of the kidney in edema formation appeared to offer several manifest advantages. If, indeed, the impression of various workers is correct that the kidney is implicated in the events leading to fluid retention, possibly in a prime role, then it should be determinable whether such intervention derives from the process of filtration or of reabsorption, and whether it is autonomous or responsible to some other agent.

Assuming that the serum sodium values* represent unbound sodium in these patients as in the normal, then the sodium filtered daily may be readily calculated and will be found to average 22,381 meq. in the case of E.W., and 9,438 meq. in that of W. S.† Comparison of these values with the approximate intake daily of 44 milliequivalents shows a disparity so impressive as to discourage the attempt to relate failure of excretion to failure of filtration since the latter function has with respect to the major tissue electrolytes such a wide margin of safety. The effective renal blood flow is seen to be within normal in E. W. and reduced by approximately 50 per cent in W. S.; it is evident that renal impairment as indicated by a reduced filtration rate and blood flow is not invariably associated with cardiac decompensation.

It is likewise noteworthy that impairment of the kidney would be expected to diminish renal working capacity. The demonstrated reabsorption of excess quantities of sodium, hence performance of increased osmotic work, indicates the reverse situation. It seems plausible to hypothesize, therefore,

*Plasma sodium and chloride concentrations for E. W. were 139 meq. and 102 meq. respectively. Those for W. S. at time of discharge were 119 meq. and 107 meq. The low sodium level in the latter case may have been associated with the existing secondary cirrhosis.

†Filtration rate (ml./min.) × serum Na^+ (meq./ml.) × 1440 = meq./24 hours.

that the stimulus to the excessive reabsorption of sodium is specific and that it depends upon physiologic factors, chemical or humoral, rather than upon an injured or invalid kidney.

The behavior of sodium with the administration of mineral diuretics as indicated in Tables I and II is predictable in terms of primary response: W. S. is seen to increase urine output somewhat and, concomitantly, sodium output with potassium chloride, ammonium chloride, and potassium acetate. With the first two substances in particular, the sodium increase is many times greater than that of water, and, notwithstanding the administration of the chloride salt, the chloride output is less sharply increased than is the sodium. In no case did the diuretic effect last more than two to four days, although the medication was continued for several more days.

The brief duration of diuresis is an observation of clinical importance as well as physiologic interest. If potassium chloride, for example, is capable of augmenting the urine volume for so short an interval of time, it could well be argued that a rotation of mineral salts might be adopted in the management of chronic cardiac decompensation.

In both patients the administration of a mercurial diuretic resulted in a urine output of approximately twice that of the control levels, but the increase in sodium output was even more marked. The release of salt and water by mercurial preparations is by no means a novel observation, but the absence of significant changes in other electrolytes is a matter of considerable theoretic interest. If the augmented absorption of sodium and, to a lesser degree, chloride were a function of a factor so nonspecific as renal blood flow, it is difficult to conceive of that factor as being abolished by the administration of mercury, to which a marked stimulation of renal circulation could hardly be attributed.[21]

Turning now to the excretion of potassium as shown in Tables I and II, only minor fluctuations are observed which, with those of ammonia, probably reflect shifts in cation-anion balance. A powerfully alkalinizing effect is noted in the urine under the influence of potassium acetate, a result which serves a useful purpose in clinical practice where alkalinization by sodium salts might be undesirable. It is also of interest that when the potassium intake is elevated by the ingestion of 100 millimols of potassium salt, a positive balance is established and a considerable portion of the element is held in the body.

The values for ammonia in the urine show the normal response to the ingestion of ammonium chloride. As in Gamble,[7] Chart 29, an elevation follows that of chloride and is prolonged beyond the chloride. A tendency to inverse proportionality with sodium and potassium can be traced, compatible with the classical theory of ammonia as a sparer of fixed base. This interpretation becomes open to question in the study of hepatic cirrhosis presented in the following paper. Organic acids found in the urine underwent virtually no changes throughout the period of the experiments. The urine calcium, magnesium, sulfate, and phosphate offer no particular grounds for discussion within the framework of the present study.

The described shifts in electrolyte components should be considered as metabolic renal function tests. As such they reveal no suggestion of renal incompetence. The probability is strong, therefore, that a kidney which is able to maintain acid-base balance, preserve blood levels, and synthesize ammonia, is retaining sodium in response to factors acting upon it and not through incompetence.

Considering now the situation in terms of fluid compartments, the clinically visible increase in extracellular volume characteristic of cardiac decompensation is analogous to the situation described by Stewart and Rourke[22] in which large infusions of isotonic sodium chloride are given daily for four days and the extracellular fluid is thus increased by approximately 80 per cent of the original volume. Examining the data reported by these investigators, we find that at the end of three days the positive fluid balance has been reversed and the urine output exceeds the water intake by almost 1 liter. The sum of the excreted sodium and chloride liberally exceeds the intake after the first twelve-hour period, and the ratio of sodium to chloride in the urine never drops below 0.89. The plasma values are not extraordinary. If it is objected that the increased venous pressure present in cardiac decompensation could not have operated in Stewart's normal subject, it might be pointed out that this situation was well simulated by the forced increase in plasma volume resulting from the daily administration of 6 to 7 liters of 0.9 per cent sodium chloride. If we may then concede that the cases are comparable with respect to the fluid compartments, we are in a position to appreciate the disturbance in excretory water and electrolyte pattern revealed in the decompensated state. It appears evident either that the normal pituitary control of fluid volumes and electrolyte concentrations is defective in the cardiac patient or that the kidney is not able to respond normally to such control.

With respect to the second possibility, namely, tubular decompensation with respect to electrolyte control, the output of individual ions other than sodium, and to a lesser degree chloride, shows no renal defect, and the responses to sharp alterations of ionic load and acid base balance indicate no deviation from normal behavior (Gamble, Charts 29-30). The data here presented might be summarized, then, by the hypothesis that the condition of heart failure with edema consists of a gross enlargement of the extracellular fluid volume, which enlargement somehow fails to elicit the normal hemostatic factors acting on renal tubules. The tubules are behaving, in fact, as normal tubules in the presence of a decreased extracellular fluid volume.

SUMMARY AND CONCLUSIONS

Two patients with rheumatic heart disease and congestive failure were studied for a period of three to four weeks by quantitative analysis of all urine electrolytes. The determinations were carried on daily, and the effects of various diuretic salts were evaluated in terms of fluid balance and urine electrolyte pattern.

Estimations of renal filtration rate and blood flow were made by the clearance of mannitol and PAH. In one patient both the filtration rate and the effective blood flow were reduced to approximately 50 per cent of normal. In the second, both functions remained well within normal range.

The urine electrolyte pattern of patients in heart failure revealed a constant and specific characteristic, namely, an absolute reduction in sodium excreted, with a resulting diminution in the ratio of sodium to chloride.

The reduction in the sodium-chloride ratio was not associated with any other constant disturbance in electrolyte excretion.

Various mineral salts were effective in promoting diuresis, but this effect was of brief duration. Outputs of water and sodium which approximate the control levels were obtained in spite of the continued administration of the diuretic.

Administration of a mercurial diuretic tended to abolish sodium retention and to increase sodium excretion to a much greater degree than it did that of water.

In cardiac edema the glomerular filtration rate may be reduced or it may be maintained within normal limits.

It is proposed that the selective activities described by the data are evidence of normal response by the renal tubules to disturbances involving the regulation of fluid compartments.

We wish to express our sincere appreciation to Professor C. J. Farmer for his generous support and advice in this study.

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ELECTROLYTE PARTITION IN PATIENTS WITH EDEMA OF VARIOUS ORIGINS

Qualitative and Quantitative Definition of Cations and Anions in Hepatic Cirrhosis

Edith B. Farnsworth, M.D., and Jacob S. Krakusin, M.S.
Chicago, Ill.

With the Technical Assistance of Lora Belle Hughes, B.A., M.T. (ASCP)

WE HAVE previously indicated the probability of sodium retention of a specific nature occurring in congestive failure and to an even greater degree in hepatic cirrhosis.[1] With respect to cardiac failure we have described the renal dynamics of salt and water retention in terms of the clearances of mannitol and PAH and the quantitative evaluation of urine electrolytes. An abnormal urinary electrolyte pattern was demonstrated which was characterized by sodium retention and a reduction in the ratio of sodium to chloride.[2] The purpose of this report is to demonstrate the qualitatively similar but quantitatively even greater retention of sodium, and to some extent of chloride, in hepatic cirrhosis.

METHODS

Two patients were selected in whom the clinical diagnosis of Laënnec's cirrhosis was supported by liver biopsy. Both were men, 52 and 54 years of age, respectively; jaundice was moderate and ascites was massive. After several days on a general diet, the patients were placed on a diet containing a maximum of 1.5 Gm. daily of sodium chloride. Mineral and mercurial diuretics were administered as indicated in Tables I and II. Paracentesis was done once or twice on each patient, and the electrolyte concentration of the ascitic fluid was determined. The cation-anion balance was quantitatively itemized from daily twenty-four hour collections of urine which were preserved under toluene and refrigerated. Analytic methods employed in the determination of ammonia, magnesium, calcium, chloride, phosphate, sulfate, and organic acids are described in the preceding paper.[2] Sodium and potassium values were determined by the Beckman flame photometer.

A few data were included from a third patient, B. G., of the same sex and age group, in whom complete collections were difficult because of incontinence and illiteracy. The urine values are therefore scattered. The data are included because of the more regular blood studies available.

RESULTS

The excretion of sodium was found to be exceedingly low with all dietary loads. The urinary suppression of sodium, regardless of daily salt ingested, was even more refractory to diuretic treatment in these patients than in the patients in congestive failure reported in the foregoing paper.

Fluid output was also consistently low, and the administration of mercurial diuretics resulted only rarely in a slight diminution in body weight, in contrast with the effect in heart failure.

From the Departments of Medicine and Chemistry, Northwestern University School of Medicine.

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TABLE I. URINE ELECTROLYTES (IN MEQ./24 HOURS); PATIENT D. E.

DATE (1947)	INTAKE	RX	OUTPUT	PH	NA^+	K^+	NH_4^+	CA^{++}	MG^{++}	CL^-	$HPO_4^=$	INORGANIC $SO_4^=$	ORGANIC ACIDS	WT.
Nov. 7	3320	General diet	750	6.0	1.1	20	33	5.5	3.0	31	6	22	22	187½
8	2380	General diet	805	5.9	1.3	29	55	7.1	2.4	36	25	17	27	191½
9	2500	General diet	1560	6.0	0.9	42	30	4.9	6.4	37	22	13	28	
10		Low salt, Hg†	1500	6.1	26	61	49	3.2	2.7	51	45	24	28	
11	2364	Low salt	2040	6.1	44	101	23	8.8	9.2	101	24	16	46	193
12		Low salt, Hg	1765	6.1	0.7	40	20	2.0	6.9	19	18	16	25	192
13	2840	Low salt	1285	6.3	12	62		8.8	10.4	42	14	16	23	191½
14	2620	Low salt, Hg	1040	6.2	2.0	38	22	1.6	7.5	12	9	14	21	192
15	2500	Low salt	1300	6.4	1.5	33		4.4	12.5	11	14	9	19	194
16	2180	Low salt, Hg	1165	6.3	1.2	26	16	1.5	6.7	6	23	12	32	194
17	2780	Low salt‡	1720	6.9	28	88	12	1.3	17.3	57	21	11	29	193
18	1640	10 Gm. potassium acetate, Hg	1450	7.4	1.7	109	6	2.2	5.8	4	28	16	27	194
19	2380	10 Gm. potassium acetate	2665	7.2	45	111	9	3.6	10.4	77	6	10	21	195
20	2560	10 Gm. potassium acetate, Hg	1580	7.4	3.5	85	7	8.0	5.6	5	14	11	21	196
21*	2080	10 Gm. potassium acetate	1590	7.4	14	115	5	2.1	5.5	17	37	9	37	176
22	2850	10 Gm. potassium acetate, Hg	1880	7.4	4	122	19	1.7	10.3	8	30	15	32	179
23	2430	10 Gm. potassium acetate	2215	7.3	17	121	6	2.7	8.9	23	25	14	27	180
24	2410	10 Gm. potassium acetate, Hg	1715	7.4	7	111	6	2.0	7.6	11	27	15	27	179
25	2080	10 Gm. potassium acetate	1500	7.3	15	120	4	3.3	10.1	37	23	10	30	177
26	2800	10 Gm. potassium acetate, Hg	1880	7.3	9	120	4	1.3	6.2	8	25	16	39	179
27	1860	10 Gm. potassium acetate	1720	6.8	21	67	8	4.5	11.5	35	23	12	25	180
28	2040	8 Gm. KCl, Hg	1700	6.9	6	59	25	2.0	9.8	6	17	13	36	181
29	2360	8 Gm. KCl	1780	6.8	7	85	11	3.5	10.7	36	14	16	37	182
30	3240	8 Gm. KCl, Hg	1840	6.8	1.7	61		4.1	10.5	9	15	13	25	182¼
Dec. 1		12 Gm. NH_4Cl	3590	6.1	262	158	28	8.4	9.9	395	8	13	29	182
2	3120	12 Gm. NH_4Cl, Hg	1300	5.9	22	40	59	8.9	11.3	78	2	25	30	180
3	3120	12 Gm. NH_4Cl	3765	6.1	284	117	66	16.0	15.6	457	18	16	34	181
4	3840	12 Gm. NH_4Cl, Hg	2340	5.7	82	59	106	10.3	8.4	204	31	24	42	177
5	2370	12 Gm. NH_4Cl	1185	6.0	9	27	91	5.3	6.3	66	16	45	24	176
6	2520	No Medication	1425	6.0	3	49	92	7.4	5.5	57	22	54	28	182
7	2460	No Medication	2085	6.2	3	72	125	11.5	18.5	89	61	49	29	179¾
8	2860	No Medication	1640	6.2	73	116	161	7.5	5.2	277	17	54	32	178
9	2380	No Medication												

*Paracentesis.

†10 mg. 2-pal Mercuhydrin [illegible]

TABLE II. URINE ELECTROLYTES (IN MEQ./24 HOURS); PATIENT J. S.

DATE (1947)	INTAKE	RX	OUTPUT	PH	NA^+	K^+	NH_4^+	CA^{++}	MG^{++}	CL^-	$HPO_4^=$	INORGANIC $SO_4^=$	ORGANIC ACIDS	WT.
Nov. 9	1100	General diet	600	5.8	1.7	38	30	0.9	2.5	16	18	21	21	148¼
10	1575	General diet	550	5.7	0.6	33	27	0.9	3.4	6	24	21	21	
11	1400	General diet	690	5.9	7	54	37	1.0	1.0	26	14	26	21	
12	1650	Low salt	350	6.0	2.2	23	32	0.5	1.1	7	8	13	13	
13	2380	Low salt, Hg†	1465	5.0	81	65	33	9.9	11.9	182	9	12	15	149¾
14	2190	Low salt	765	6.0	30	51	15	3.3	3.2	49	23	27	19	145½
15	2250	Low salt	350	5.8	0.3	14	6	0.7		3	8		14	152
16	2030	Low salt, Hg	1050	5.6	34	66		7.9	18.9	106	11	22	23	151
17	1210	Low salt‡	1020	6.0	4	33	41	2.0	2.0	23	18	29	28	151
18	1490	8 Gm. KCl, Hg	1345	5.7	22	61	23	5.4	9.6	73	14	20	28	155¼
19	2140	8 Gm. KCl	720	6.3	0.7	33	19	0.8	0.2	3	5	16	29	155¼
20*	2150	8 Gm. KCl, Hg	1930	6.4	41	104	27	8.1	8.2	111	6	16	38	157
21	2040	8 Gm. KCl	1340	6.5	1.0	45	23	1.3		5	8		30	139
22	1100	8 Gm. KCl, Hg	1770	6.1	28	143	35	7.3	7.2	123	16	20	29	140¾
23	1540	8 Gm. KCl	2045	6.2										141½
24	2290	8 Gm. KCl, Hg	310	6.6	2.5	39	9	1.4	2.2	17	8	2	9	142
25	1580	10 Gm. potassium acetate	1555	6.3	0.8	56	9	1.0	1.0	4	17	16	22	144½
26*	2500	10 Gm. potassium acetate, Hg	1290	5.9	4.6	97	15	2.5	2.2	54	5	17	28	144¾
27	1640	10 Gm. potassium acetate	985	6.0	1.3	49	16	0.9	1.7	8	10		24	133½
28	1900	10 Gm. potassium acetate, Hg	1690	6.5	0.3	84	20	6.2	6.0	21	15	34	37	135¾
29	1600	10 Gm. potassium acetate	1915	6.8	1.0	77	21	1.4	3.5	27	6	26	36	137½
30	1450	10 Gm. potassium acetate, Hg	2750	5.5	34	103	33	7.4	7.8	158	0.6	14	28	139
Dec. 1	2710	12 Gm. NH_4Cl	3350	5.7	8	98	35	4.4	3.7	51	27	21	40	137½
2	2840	12 Gm. NH_4Cl, Hg	3015	6.1	4	120	47	5.6	6.1	156	6	19	30	
3		12 Gm. NH_4Cl												
4	1710	12 Gm. NH_4Cl, Hg	550	5.6	36	18	12	2.7	1.3	74	3	4	6	136
5	2040	No medication	210	5.8	0.3	3.0	25	1.2	3.7	5	6		8	
7	1215	No medication	570	6.0	2.2	6	37	2.0	2.9	7	7	23	14	134
8	2090	No medication	590	6.1	1.4	5	23	0.9	2.3	6	5	9	9	135¼
9		No medication	1000	6.1	0.4	11	37	1.3	4.8	5	8	23	15	

*Paracentesis.

†Hg, 2 ml. Mercuhydrin.

‡The low salt diet was maintained for the duration of the experimental period.

Chloride output was also exceedingly low and tended to be lower than in heart failure. With relation to sodium output, however, the diminution was less extreme, hence the sodium-chloride ratio remained lower than in normal subjects or in congestive failure. The use of mercurial diuretics caused a greater chloride than sodium excretion. This increase in chloride output was in turn frequently associated with an increase in urine potassium.

The excretion of ammonia in response to the administration of ammonium chloride was very different in the two subjects: D. E. showed the typical delayed but protracted increase in urine ammonia (Gamble, Chart 29),[3] and J. S. appeared unable to synthesize ammonia.

Administration of potassium acetate, 100 meq. daily, was associated with a low ammonia output, in the cirrhotic subjects as in the cardiac patient to whom the medication was given.

Sulfate excretion tended to be low, a finding which is presumably to be correlated with interference with metabolism of sulphur containing amino acids in the liver.

The excretions of calcium and magnesium were variable in both patients. The determinant of these variations appeared to be chloride rather than phosphate excretion.

DISCUSSION

On inspection the data resemble rather closely those derived from the patients in cardiac failure presented in the preceding paper. The initial control periods, without dietary restriction of salt, indicate a retention of sodium, chloride, and water. These positive balances persist notwithstanding the imposition of electrolyte loads by mineral diuretic medication and in spite of the frequent administration of mercurial diuretics. These facts parallel the findings in congestive failure, and establish the fact that in Laënnec's cirrhosis sodium retention rather than hypoproteinemia is a major determinant of ascites and edema. Here again, in cirrhosis as in cardiac failure, the pattern is not one solely of filtration of plasma water and electrolytes through the capillary wall, but a more complicated sequence in which the reabsorptive activity of the renal tubules plays a substantial part. As in the cardiac subject, the primary or secondary order of such renal participation becomes a concern of fundamental importance.

While the retention of salt and water lends similarity to the two entities, certain sharp differences appear. First, the urinary sodium concentrations tend to be lower in our three patients with cirrhosis than in congestive failure; indeed, during the control period on a general diet all patients showed a total absence of sodium when the gravimetric procedure was attempted on unconcentrated urine. Although the absolute chloride content was also markedly reduced, it was still high enough to yield a sodium-chloride ratio which was decidedly lower than those found in cardiac patients. The response to mercurial diuretics indicates a more striking divergence. Figs. 1 and 2 show only minimal variations in urine volume during the administration on alternate days of Mercuhydrin, and the body weight appears to be unaffected by medical diuretics.

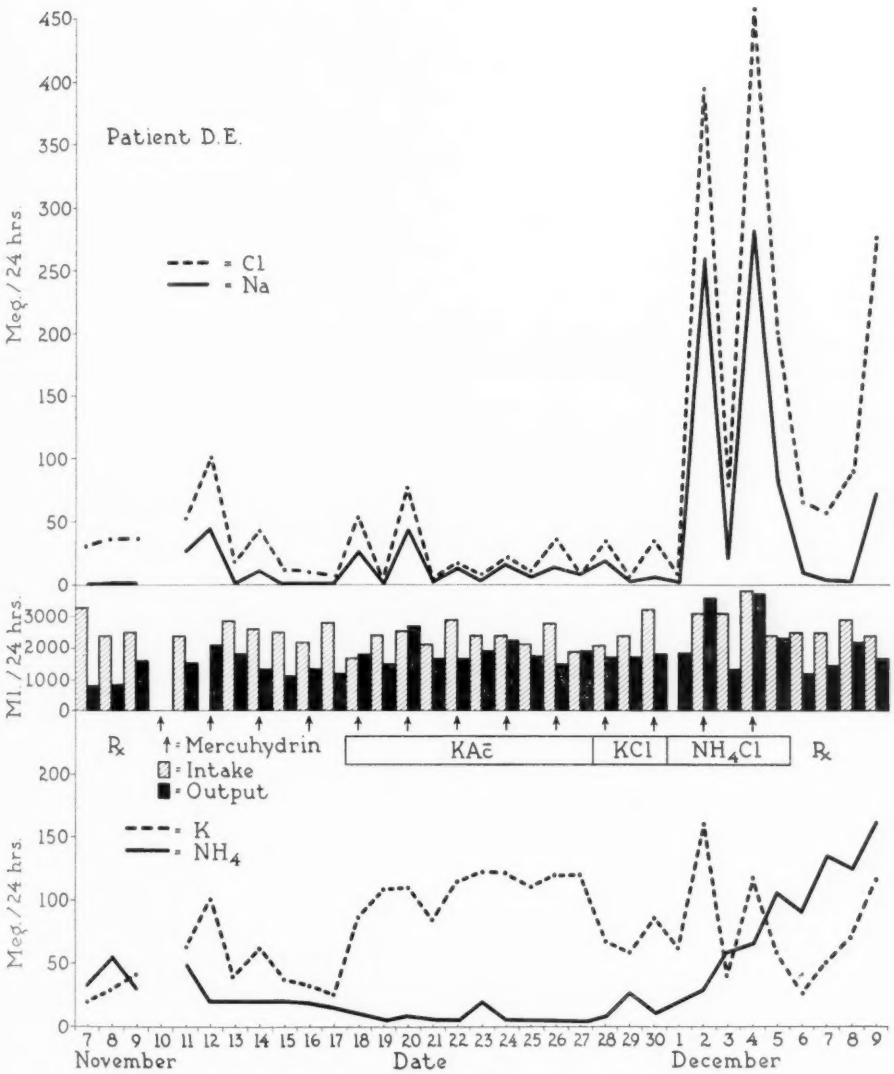

Fig. 1.—D. E.: electrolyte and water excretion with diuretics.

The excretion of sodium is mildly augmented by mercury, but, contrary to experience with patients in heart failure, the effect on chloride is consistently more marked, and hence the ratio sodium-chloride is not restored to normal at any time. These observations pose several questions: firstly, are they the results of incompetent kidneys; second, are they due to abnormalities of electrolyte concentration in one or all of the fluid compartments.

With respect to the functional integrity of the kidneys, the clearance rates of mannitol and PAH are given in Table III along with the blood chemistry values. Although substantially below the normal, they can hardly be taken to indicate renal decompensation. The fluctuation of urine values of ammonia in

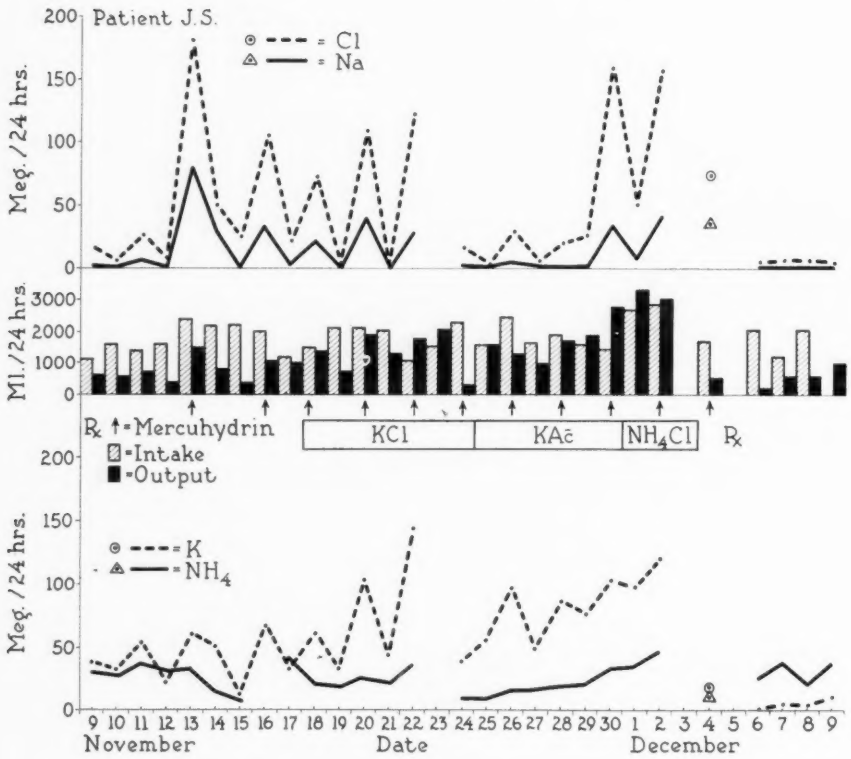

Fig. 2.—J. S.: electrolyte and water excretion with diuretics.

response to ammonium chloride, potassium in response to potassium salts, and sodium in response to ammonium chloride are in themselves renal function tests and offer evidence that the kidneys are not in default. Attention should be called parenthetically to the differences in the two records toward the end of the experimental period. D. E. tolerated the ammonium chloride, 220 meq. daily, for five days, and underwent a dramatic diuresis of electrolytes; whereas J. S. became comatose after three days on similar amounts of ammonium chloride and the medication was discontinued when the plasma CO_2 reached 17 meq. per liter. Although the urine pH of this patient did not decrease below that of D. E. under the same conditions, J. S. appeared to be unable to synthesize ammonia and continued to put out a small volume of urine, poor in electrolytes, in spite of clearance values almost identical with those of D. E. The reason for this discrepancy is probably to be found in the poor nutritional condition and extreme anorexia which characterized his hospital course, in contradistinction to D. E. who ate heartily throughout the period of observation.

The second question which we have proposed concerns the volume and electrolyte composition of the fluid compartments in cirrhosis. In our experience patients with cirrhosis have shown normal or only slightly lowered plasma sodium while on a general diet; reduction in intake has resulted, in several instances, in markedly reduced levels. At all plasma concentrations, however, the

TABLE III. RENAL FUNCTION AND PLASMA AND ASCITIC FLUID VALUES; PATIENTS D. E. AND J. S.

	CLEARANCES (ML./MIN.)		PLASMA							ASCITIC FLUID (MEQ./LITER)			
			MEQ./LITER				GM./100 ML.						
	MANNITOL	PAH	NA^+	K^+	CL^-	$HPO_4^=$	TOTAL PROTEIN	ALBUMIN	GLOBULIN	NA^+	K^+	CL^-	$HPO_4^=$
D. E.													
Nov. 17	74	415	131	5.8	99	2.8							
21													
25										133	5.7	102	1.6
J. S.							6.6	3.0	3.6				
Nov. 12							5.9	2.8	3.1				
20	61	496	124	6.3	3.1	106				137	6.5	107	3.0

TABLE IV. ELECTROLYTE PARTITION IN PLASMA AND URINE; PATIENT B. G.

DATE	INTAKE	RX	URINE (MEQ./24 HR.)							WT.	PLASMA (MEQ./LITER)			
			OUTPUT	PH	NA^+	K^+	NH_4^+	CL^-	$HPO_4^=$		NA^+	K^+	CL^-	$HPO_4^=$
May 20		General diet								146	141	4.9	105	2.6
24	1700	General diet	160	5.9	0.1	62	18	7	8	150				
25	1700	General diet	315	5.8	0.3	23	37	24	14	151¼				
26	2040	General diet	420	5.8	6	48	46	35	20	154				
27	1250	General diet	360	6.0	0.6	29	41	24	15	156¼				
June 1	1500	Low salt	570	5.8	0.3	15	58	15	16	168	131	4.0	105	2.5
2*	1300	Low salt	310	5.9	0.6	29	32	6	10					
8	2500	5% Glucose in water	2400		0.5	7		7	10	152				
9	5550	5% Glucose in water	1930		0.8	23		8	11					
10	3140	5% Glucose in water	2300	6.8	0.4	17		8	9	152	120	4.2	99	2.1

*Paracentesis.

urine excretion of sodium was almost entirely suppressed. The data given in Tables III and IV show normal concentrations in plasma and ascitic fluid of sodium, potassium and chloride in the case of D. E., with similar values for J. S. with the exception of the plasma sodium of 124 meq. Plasma sodium values for B. G. declined from 141 meq. on a free diet to 120 meq. on a low-sodium diet. At those two levels the urine output of sodium was 0.1 meq. and 0.4 meq. respectively. The volume of the extracellular fluid compartment, however, was grossly enlarged, as evidenced by the massive dependent edema as well as ascites of all patients. Here again a situation exists which would normally elicit a spontaneous diuresis, since it is the major function of the renal excretory mechanism to defend not only the concentration but the volume of the extracellular fluid. With respect to salt and water, then, the kidneys are behaving as if the body were dehydrated. The paradox is essentially the same as was found to exist in the state of heart failure, only to a more marked degree.

The findings which we have described support the impression expressed by recent investigators[4, 5, 6] that abnormalities in water distribution in cirrhosis cannot be accounted for solely on the basis of plasma protein deficiency and diminished colloid osmotic pressure.

CONCLUSIONS

The urinary electrolyte pattern in hepatic cirrhosis is similar to that in cardiac failure. The renal retention of salt and water, long recognized in heart failure, is present to a more marked degree in hepatic cirrhosis.

As in heart failure, the mannitol and PAH clearances may be somewhat reduced, but the metabolic functions of the kidneys are essentially unaffected, and other urinary electrolytes exhibit the normal responses to variation in electrolyte intake.

Water and salt diuresis was minimal after administration of mercurial diuretics. One instance is shown, however, in which a lavish diuresis of electrolytes was initiated by Mercuhydrin.

In addition to the hydrostatic factors operating in cirrhosis, the genesis of edema and ascites in this condition is also attributable to a retention of sodium and water. This urinary suppression of sodium is independent of the plasma concentration. Since the competence of the kidneys has been demonstrated by clearance determinations of mannitol and PAH as well as by quantitation of urine electrolytes, the hypothesis is suggested that the stimulus to salt and water retention is specific and that the same stimulus is probably acting in both diseases.

We extend our warm thanks to Dr. A. C. Corcoran for his help in the preparation of this paper.

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ON THE EFFICACY AND SAFETY OF GLYCINE ADMINISTERED BY VEIN

GEORGE E. COLLENTINE, JR., B.S., M.D.
MILWAUKEE, WIS.

THE current rapidly increasing use of amino acid preparations by the intravenous route has suggested a more specific study of certain effects of the simplest of these compounds, glycine, when administered by this method. Although not a significant constituent of the available commercial products, since these are principally acid or enzymic hydrolysates of casein, which contains little glycine, this amino acid deserves consideration.

Because it is readily synthesized in apparently adequate amounts by the normal human body, glycine falls into the classification of "nonessential" amino acids—a connotation unfortunately accepted by many to be synonymous with "unimportant," which may explain the lack of attention to this compound in the field of parenteral amino acid preparations. Glycine, however, is a component of many of the body proteins (collagen is rich in glycine); its roles in the syntheses of such nonprotein substances as creatine,[1] glycocholic acid, glutathione,[2] the protoporphyrin portion of hemoglobin,[3] and uric acid[4] have received widespread attention and confirmation in isotope studies during the last few years. In each instance the ingestion of glycine tagged with N^{15} has been followed so quickly by the appearance of labeled molecules of these products as to indicate the direct participation of this amino acid in their syntheses. Glycine, moreover, has long been known to take a leading part in the detoxication mechanisms applied by the body against a large number of potentially harmful products of metabolism. Representative of this action is the conjugation of benzoic acid, an oxidation product of many toxic aromatic compounds, with glycine to form hippuric acid.

The Quick test of liver function is based on the relationship between the functional state of that organ and the capacity for synthesis of hippuric acid from ingested benzoic acid. It has been shown that supplying extra glycine by mouth during the test will in some instances appreciably increase the hippuric acid output even when it is seriously diminished in liver disease.[5] Thus it is evident that the actual ability of the liver cell to bring about the conjugation may not be impaired, but that the rate of synthesis of glycine is the critical consideration. Depression of this rate occurs in normal pregnancy to the extent of 15 per cent below normal,[6] and in toxemias of pregnancy and other conditions the ability to detoxicate is much more seriously impaired.[7]

Here again isotope studies have shed some light upon the mechanism involved. Simultaneous feeding of sodium benzoate and an excess of labeled

From the Department of Biochemistry, Marquette University School of Medicine.

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glycine resulted in the utilization of exogenous amino acid for about one-third of the hippuric acid synthesis.[8] The body must have supplied the remaining two-thirds required for the detoxication. Very little is known of the source or precursors of this endogenous supply. The observation of Christensen and co-workers that the ingestion of sodium benzoate is followed by a depression of the fasting blood glycine level in human beings[9] has been confirmed in this laboratory. From this it would seem that the body maintains its glycine reserves freely mobile in the blood stream, and depends upon rapid synthesis to meet specific demands for detoxication and other purposes.

Recently, Gubner and associates[10] have employed the specific dynamic action of glycine to bring about some conceivably desirable changes in peripheral blood flow. Their work was based on the correlations that have been established between the metabolic rate and cardiac output and peripheral blood flow.[11, 12] By means of skin temperature recordings, oscillometric readings, plethysmographs, and oxygen consumption determinations they have shown that the increased heat production and oxygen consumption that follow the ingestion of glycine are accompanied by a maximal increase in circulation to the extremities. The skin temperature changes produced are equivalent to those obtained with nerve block and are consistently greater in duration and degree than those caused by alcohol. The presence of peripheral vascular disease in some of their subjects did not alter the response.

This considerable multiplicity of important metabolic functions, viewed in the knowledge that the rate of synthesis of glycine is depressed in a variety of conditions, lends plausibility to the idea that reasonable indications may exist for the supplying of glycine by veins when enteral nutrition is precluded. The studies to follow were undertaken to investigate some effects of glycine administered intravenously.

EXPERIMENTAL

For the determination of glycine concentrations in blood and urine, the specific colorimetric micromethod of Alexander, Landwehr, and Seligman[13] was employed, with the only difference that a Cenco photelometer was used instead of the suggested Evelyn or Klett instruments. This method depends upon the distillation-conversion of all free glycine into formaldehyde by the action of ninhydrin, the resulting formaldehyde being allowed to react with chromotropic acid to produce a measurable color. Metabolism determinations were made with a standard waterless respirometer.

I. Two female dogs previously fasted for fourteen hours were given infusions of 5 per cent or 10 per cent glycine in physiologic saline solution by simple intravenous drip, the rate of flow being regulated to take thirty minutes. Blood samples were withdrawn immediately before the infusion, immediately afterward, then one and two hours later. Catheterized urine specimens were obtained for the hour preceding administration of glycine and at hourly intervals after the drip began. Two doses were employed: one amounted to 0.5 Gm. of glycine per kilogram of body weight and the other to 1.0 Gm. per kilogram. With the latter dose, both dogs showed signs of distress such as bradycardia, extrasystoles, excessive salivation, and vomiting at the conclusion of the infusion. These signs diappeared within the next half hour, and on a subsequent occasion one of the dogs tolerated this dose without apparent ill effect. Table I is a record of urinary output of glycine during four of the preceding experiments on one of the dogs.

TABLE I. HOURLY OUTPUT OF GLYCINE IN URINE OF A 10-KILOGRAM DOG, FOLLOWING INTRAVENOUS INFUSION OF GLYCINE IN SALINE

HOUR	100 ML.	5% GLYCINE	100 ML.	10% GLYCINE
1	0.2 mg.	0.2 mg.	0.1 mg.	0.2 mg.
2*	307.3	283.5	1,572.5	1,252.8
3	18.0	21.0	109.2	146.3
4	3.6	4.2	21.0	38.8
Total	328.9 mg.	308.7 mg.	1,702.7 mg.	1,436.9 mg.

*Infusion begun at end of control hour 1; duration, thirty minutes.

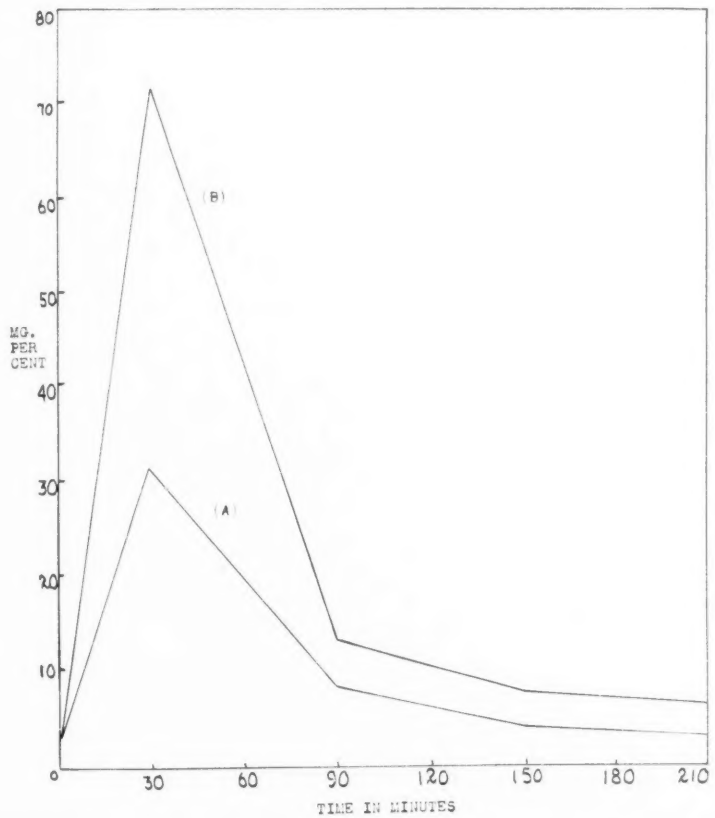

Fig. 1.—Blood concentration curves in dog and human being (see text).

II. Eight normal adults, fasting for twelve hours, were given a total of about twenty intravenous doses of glycine ranging from 12 to 16 Gm. on a basis of 0.2 Gm. per kilogram of body weight, with the method of administration similar to that in the previous experiments. One subject was given a dose of 30 Gm. (0.4 Gm. per kilogram), and at the end of the half-hour injection he noted a feeling of warmth and tingling in the extremities, some increased salivation, slight nausea and lightheadedness, all of which passed within a half hour. No noteworthy subjective manifestations were mentioned by subjects given the smaller dose, except the feeling of tingling, which was inconstant.

Blood specimens were taken immediately before and after the infusion and at one, two, and three hours thereafter. Duplicate tracings to determine basal metabolism were made before administration of glycine, and single tracings were made before each blood withdrawal.

It was soon noted that the curves of blood concentrations obtained on these rather arbitrarily selected doses in dogs and human beings showed striking similarity. Indeed, when average curves for each series were drawn, they could be superimposed for presentation. In Fig. 1, curve *A* represents the averagee of six closely comparable curves obtained in dogs with the smaller doses (0.5 Gm. per kilogram) and of a dozen or more similar curves

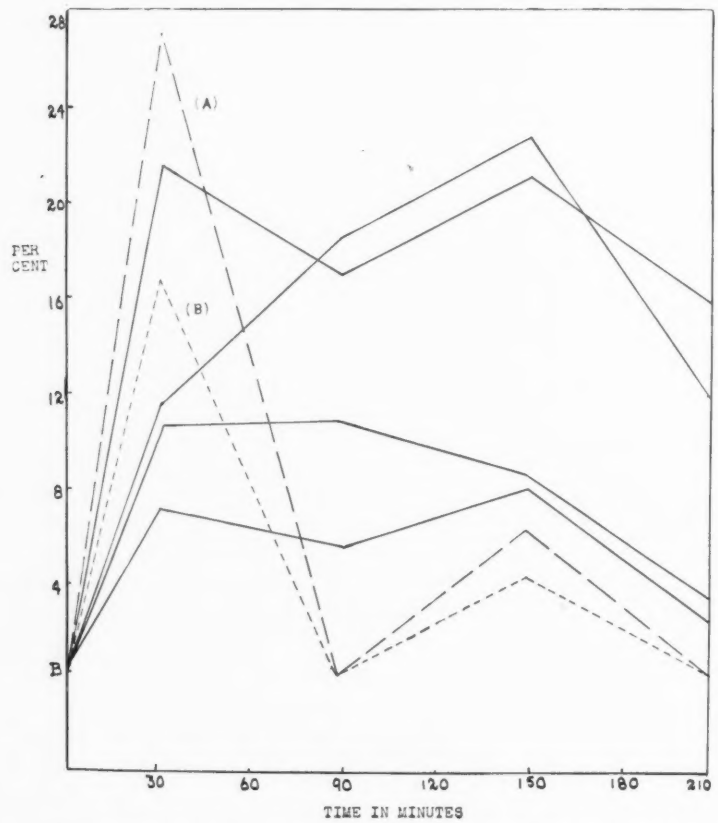

Fig. 2.—Per cent increase in oxygen consumption: (*A*), following dose of 0.4 Gm. per kilogram; (*B*), same subject as (*A*), after dose of 0.2 Gm. per kilogram; others, after latter dose.

from human beings given 0.2 Gm. of glycine per kilogram. Curve *B* was obtained with the doses that produced untoward symptoms in both dog and human being (1.0 and 0.4 Gm. per kilogram respectively).

In Fig. 2, some curves showing per cent of increase in rate of oxygen consumption above the basal level are presented. A control experiment, using only intravenous saline without glycine, was performed with one student who acted as subject for the glycine experiment on six occasions. This subject showed a perfectly constant rate of metabolism throughout the control test period, but responded to the addition of glycine with increases similar to those presented.

TABLE II. HOURLY OUTPUT OF GLYCINE IN URINE OF A 75-KILOGRAM MAN, FOLLOWING INTRAVENOUS INFUSION OF GLYCINE IN SALINE

HOUR	150 ML. 10% GLYCINE (0.2 GM./KG. B.W.)	300 ML. 10% GLYCINE (0.4 GM./KG. B.W.)
1	2.0 mg.	2.0 mg.
2*	1,722.0	3,997.6
3	163.5	347.8
4	28.4	35.1
5	4.2	10.0
Total	1,918.1 mg.	4,390.5 mg.

*Infusion begun at end of control hour 1; duration, thirty minutes.

Urine studies on the subject who received both doses are recorded in Table II, and those on two subjects who received only the small dose, in Table III.

TABLE III. HOURLY OUTPUT OF GLYCINE IN URINE OF 70- AND 80-KILOGRAM MEN, FOLLOWING INTRAVENOUS INFUSION OF GLYCINE

HOUR	140 ML. 10% GLYCINE (0.2 GM./KG. B.W.)	160 ML. 10% GLYCINE (0.2 GM./KG. B.W.)
1	1.8 mg.	2.5 mg.
2*	1,544.4	1,696.2
3	200.7	233.9
4	24.3	31.4
5	7.5	3.5
Total	1,776.9 mg.	1,965.0 mg.

*Infusion begun at end of control hour 1; duration, thirty minutes.

III. Blood glycine concentration curves were obtained with 0.2 Gm. per kilogram dose in two patients with clinical chronic hepatitis (portal cirrhosis). Diagnosis in each case had been confirmed by punch liver biopsy and by abnormal responses to at least three different liver function tests (bromsulfalein, hippuric acid, thymol turbidity, and cephalin flocculation). From the data in Table IV it may be seen that the curves were practically identical to each other and to the curve presented as the average of all normal adults. The fasting blood levels are toward the high range of normal, but the response to the infusion is strikingly close to the average.

TABLE IV. BLOOD GLYCINE CONCENTRATIONS OBTAINED IN TWO PATIENTS WITH PORTAL CIRRHOSIS GIVEN THE 0.2 GM. PER KILOGRAM BODY WEIGHT DOSE OF 10 PER CENT GLYCINE IN SALINE; COMPARE WITH CURVE A, FIG. 1

TIME (MIN.)	BLOOD LEVELS	
	I	II
0	2.8 mg.	3.0 mg./100 ml. blood
30	32.0	31.0
90	7.6	7.8
150	5.6	5.4
210	5.0	4.5

COMMENT

The intravenous doses of glycine required to produce signs of distress in dogs (1.0 Gm. per kilogram) correspond closely to those reported by Lewis,[14] Riker and Gold,[15] and Loomis and Quick.[16] The dog that showed signs from the dose on one occasion and not on a subsequent trial had a slightly lower maximum blood level on the second occasion (70.0 mg. per 100

ml. as compared with 74.0 on the first). It is noteworthy that the blood concentration producing symptoms is similar in the dog and man, although the dose per unit of body weight required to produce them is two and one-half times greater in the dog. In view of the fact that all subjects tolerated the 12 to 16 Gm. (0.2 Gm. per kilogram) intravenous doses, even in the short injection time employed, it may be said that this represents a safe dose.

Christensen and associates[9] and Gutman and Alexander,[17] the only investigators who have published results of specific determinations of glycine in blood by the method used here, have reported fasting levels slightly lower than those obtained in these experiments. Initial levels in their subjects ranged between 1.8 and 2.3 mg., averaging 2.0, while the results presented here are based on fasting levels of 2.4 to 2.8, averaging 2.6. There was a remarkable constancy of this characteristic in individuals tested repeatedly.

The apparently "normal" blood level curves obtained in the two subjects with cirrhosis are considered an indication that a "glycine tolerance test" along these lines would have no diagnostic value in this disease, where the deficiency with respect to glycine is in the rate of supply rather than in the metabolism of the amino acid provided exogenously. In this connection, however, the work of Weichmann and Dominick on tolerance of intravenous glycine in health and diabetes is worthy of mention. They have demonstrated that following the administration of glycine the amino acid nitrogen content of serum returns within normal limits much more rapidly in healthy than in diabetic subjects.[18] Insulin caused a faster return. Duplication of these experiments, using the specific glycine determination, might produce even more clear-cut results.

Pitts has shown that of the four amino acids (glycine, alanine, glutamic acid, and arginine) the rate of renal tubular reabsorption is highest for glycine.[19] This probably accounts for the strikingly low excretion of unchanged glycine even when the blood concentration is as much as thirty times the fasting level.

The abrupt drop in the blood level, without corresponding urinary excretion, during the first hour after the infusion is an indication of the rapidity with which the glycine is metabolized. The work involved in this task of deamination and redistribution of the resulting fractions is reflected in the elevated caloric production. No previous reports of specific dynamic action of amino acids administered intravenously to human beings could be found in the literature. Inasmuch as the necessary total nitrogen metabolism studies were not undertaken, it is not possible to express the results obtained in terms that relate them to the quantity of given substance metabolized, as suggested by Peters and Van Slyke. The metabolism effect obtained here with glycine administered by vein corresponds generally in magnitude with that reported by Gubner and associates to follow oral ingestion.

Lusk showed conclusively that the increased heat production of specific dynamic action cannot be utilized for the performance of work. Nevertheless, the possibility remains that this heightened state of metabolic activity

which can be induced by the administration of a practically nontoxic, readily-available amino acid might well be desirable, for example, in medical and surgical convalescents. The associated augmentation of peripheral blood flow cannot be ignored. In any condition in which the depression of the body's ability to supply glycine is known or expected (as in preoperative fortification of the liver for biliary tract surgery), the body should profit from parenteral glycine in much the same manner as it does from glucose.

SUMMARY

Glycine plays an important role in the synthesis of body protein, creatine, glycocholic acid, glutathione, uric acid, and heme. It is apparently essential in detoxication and exhibits remarkable versatility in general metabolism. The possibility of depression of glycine synthesis in abnormal states should be recognized.

Blood and urine studies following the intravenous administration of glycine are presented to demonstrate the rapidity of its metabolism and its lack of toxicity even in high blood concentrations. Specific dynamic effect is obtained when it is given by this route.

As an adjunct to the usual parenteral protein therapy, glycine may be administered intravenously with safety and with beneficial effect when oral feeding is not possible.

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DETERMINATION OF ANTIBODY CONTENT OF LYMPHOID CELL EXTRACTS

FRANCES SPEAR, B.A., M.S.
BETHESDA, MD.

RECENT investigations[1, 2] of the role of lymphocytes in antibody formation have focused attention on the technical difficulties of serologic titration with small volumes of cells. The present work represents an investigation of the requirements of serologic technique using the small cell volume available from the lymph or lymph nodes of experimental animals.

The lymph was obtained from the efferent vessel of the popliteal node of a rabbit. The cell content of the lymph was determined using a white blood cell pipette and a standard counting chamber as used for peripheral blood examination. The total cell volume was estimated by the formula of Harris and coworkers[1] in which cell volume in milliliters equals the cell count in thousands times 0.002 times the number of milliliters of lymph. The total cell volume available in our experiments varied from 0.001 to 0.01 milliliter. It was felt that the smallest volume that could be pipetted with accuracy in this work was 0.05 milliliter. Employing the principle of "Hemolytic System Adjustments"[3] the following procedure for diluting lymph extracts was followed using 0.2 ml. pipettes graduated in 0.001 ml., and test tubes 10 by 75 millimeters.

TUBE		SALINE		FACTOR
1	0.05 ml. substance	---		1
2	0.05 ml. substance	0.05	mix, remove 0.05 ml. to control tube	2
3	0.05 ml. substance	0.1	mix, discard 0.1 ml.	3
4	0.05 ml. substance	0.15	mix, transfer 0.05 ml. to tubes 5, 6, 7	4
5	0.05 from tube 4	0.05	mix, discard 0.05 ml.	8
6	0.05 from tube 4	0.10	mix, discard 0.10 ml.	12
7	0.05 from tube 4	0.15	mix, transfer 0.05 ml. to tubes 8, 9, 10	16
8	0.05 from tube 4	0.05	mix, discard 0.05 ml.	32
9	0.05 from tube 4	0.10	mix, discard 0.10 ml.	48
10	0.05 from tube 4	0.15	mix, transfer 0.05 ml. to tubes 11, 12, 13	64

To each tube 0.05 ml. of *Salmonella typhimurium* antigen (density equal to McFarland tube number 5) was added; racks were shaken and incubated in a water bath at 37° C. for two hours, were left in the cold room overnight and read the following morning.

A comparison of this method of dilution and the usual macromethod of employing 0.2 ml. of material in serial dilution in 0.2 ml. of saline is given on the following page.

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	MACRO							MICRO							
Dil. Serum	10	20	40	80	160	320	640	10	20	30	40	80	120	160	320
1	4	3	2	+	+			4	4	4	3	±	±		
2	4	4	3	+	+			4	4	4	3	+			
3	4	4	4	4	4	+++	±	4	4	4	4	4	4	+	
4	4	4	4	+	±			4	4	4	3	2	+	±	
5	4	4	4	4	±			4	4	4	3	2	+	±	

Lymphoid cell extracts were prepared by freezing and thawing, with and without preliminary washing of the cells. After the lymph had been drawn, and the count taken, the rest of the lymph was measured into a small test tube and centrifuged at 2,000 revolutions per minute for five minutes to sediment the cells. The lymph was withdrawn with a capillary pipette and saved for testing; the cells remained in the same tube. To the estimated volume of cells, a volume of saline was added which would give sufficient material for testing with a minimal dilution factor for the cells. Lysis of the lymph cells to extract antibodies was effected by freezing with dry ice and thawing at 37° C. This process was repeated three times. A clear supernatant was obtained by centrifugation and used in the serologic test outlined.

In addition, experiments were carried out in which the lymph was divided into two portions. One portion was treated as previously outlined. In the procedure with the other, instead of extracting the cells in the saline, they were gently resuspended and centrifuged, and the saline was removed and saved for testing. The cells were resuspended in a like volume of saline and subjected to the lysing process.

In the example of results given below, the rabbit had been injected in the left foot pad with 1 c.c. of *S. typhimurium* suspension equal in density to McFarland tube number five.

Antibody titer of unwashed cells of lymph from right popliteal node	1:102
Antibody titer of unwashed cells of lymph from left popliteal node	1:70
Antibody titer of washed cells of lymph from right popliteal node	Negative
Antibody titer of washed cells of lymph from left popliteal node	1:13
Antibody titer of saline wash of right cells	1:10
Antibody titer of saline wash of left cells	1:13
Antibody titer of right lymph	1:160
Antibody titer of left lymph	1:160
Antibody titer of serum	1:480

It appears that the apparent high titer of extracts of cells of lymph may be entirely or in large part due to small amounts of lymph adherent to unwashed cells, or to the sides of the tube. Therefore these effects were investigated further.

Four tenths milliliter of a serum with a titer of 1:512 was placed in a small test tube and the serum was carefully removed with a capillary pipette. A volume of 0.05 ml. saline was delivered into the same test tube and removed with a capillary pipette, whereupon a second volume 0.05 ml. volume of saline was added. In serologic measurements the first saline wash was positive in a

dilution 1:256, and second wash was positive in a dilution of 1:12. Had the titer of the salines been calculated in reference to a hypothetical volume of cells (0.0025 ml.) which had been frozen and thawed to extract immune bodies, the titer of the first wash would have been 1:2856, the second wash, 1:324. Hence the presence of traces of fluids containing immune bodies greatly distorts titers which are computed with reference to extracts of extremely small volumes of cells.

Again multiple washes were used to eliminate the effects of traces of body fluids in an investigation of the extracts of cells of the popliteal node. The detailed procedure was as follows. The popliteal node was weighed and placed in a small beaker with a volume of saline nine to nineteen times its weight. The node was cut sufficiently to permit the release of the cells yet leave the node tissue in one minced section rendering filtration unnecessary. After a cell count had been taken, the cell saline suspension was measured into a small test tube and centrifuged (1,000 × gravity) for five minutes. The supernatant was carefully withdrawn (referred to below as wash 1) and saved for testing. Droplets of fluid observed on the sides of the test tube were removed with a clean cotton swab. Employing the same test tube, the volume of cells (computed as count in thousands × volume in milliliters × 0.0002) was resuspended in fresh saline, an amount which was a multiple of the volume of cells and gave sufficient material for testing. After this second suspension had been centrifuged, the supernatant was withdrawn and saved as wash 2.

The cells were then suspended in an amount of distilled water equivalent to half of wash 2. After the cells had been lysed through the combined effects of distilled water and alternate freezing and thawing, a volume of 1.7 per cent sodium chloride solution equal to the amount of distilled water was added. The mixture was shaken, allowed to stand, then centrifuged. The microtechnique was used to estimate the amount of antibody present in the cell extract as well as the washes. A typical result is presented.

Wash 1 titer 1:80 (proportion computed for weight of node)
Wash 2 titer 1:30 (proportion computed for volume of cells)
Cell extract titer 1:16 (proportion computed for volume of cells)

SUMMARY

The present work represents an investigation of the requirements of serologic technique using the small cell volume available from lymph or lymph nodes of experimental animals.

The experience of this laboratory indicates that especial care must be exercised in the interpretation of titers because small carryovers of fluid containing antibodies may greatly distort results if these are expressed in terms of cell volume.

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THE EFFECT OF NITROGEN MUSTARD AND X IRRADIATION ON BLOOD COAGULATION

LEON O. JACOBSON, M.D., EDNA K. MARKS, EVELYN GASTON,
J. GARROTT ALLEN, M.D., AND MATTHEW H. BLOCK, M.D.*
CHICAGO, ILL.

INTRODUCTION

ALLEN, Jacobson, and associates[1-4] reported that exposure to ionizing radiations produced a prolonged whole-blood coagulation time (Lee-White) in dogs and in human beings as a result of the appearance in the blood of an anticoagulant biologically similar to heparin. More recently Smith, Jacobson, and co-workers[5] observed a prolonged whole-blood coagulation time in five patients given therapeutic doses of nitrogen mustard (methyl-*bis*-(β-chloroethyl) amine hydrochloride). This clotting defect was identical to that reported by Allen and co-workers in the dog and human being following exposure to ionizing radiation in that the clotting time could be returned to normal both in vivo and in vitro by specific antiheparin substances such as toluidine blue and protamine sulfate. The prothrombin time and the calcium and fibrinogen blood levels were within normal limits in these subjects but each had a severe leucopenia and thrombocytopenia.

Reports by Jacobson and associates,[6] Spurr and co-workers,[7] and Block and co-workers[8] have pointed out the toxic effects of nitrogen mustard on the blood and blood-forming tissue of patients who were given this drug therapeutically. Although these authors reported pancytopenia, a prolonged bleeding time, ecchymosis, and cutaneous and mucous membrane petechiae, no alteration of significance in the whole-blood coagulation time was described.

The dosage and schedule of administration of nitrogen mustard in the five patients referred to were as follows: Two patients were given 0.1 mg. per kilogram of body weight intravenously on four consecutive days. The injections were administered twenty-four hours apart and the total dose in one was 25.4 mg. and in the other 26.8 milligrams. One patient received four injections of 0.1 mg. per kilogram of body weight at intervals of twelve hours; the total dose was 22.4 milligrams. One patient was given four injections of 0.1 mg. per kilogram at intervals of seven hours; the total dose was 20.0 milligrams. One patient was given two injections of 0.3 mg. per kilogram, each six hours apart; the total dose was 38 milligrams. The fact that a severe hemorrhagic state, terminating fatally, developed in all of these five patients led us to suspect an impurity or some intramolecular transformation in the drug or unusual sensitivity of the patient to the drug. However, other pa-

From the Biology Division of the Argonne National Laboratory and the Department of Medicine, University of Chicago.

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*Senior Research Fellow, Cancer, United States Public Health Service. Supported in part by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.

tients treated with the same lot number of the drug and with similar total dosage did not manifest this clotting defect nor has a new supply of this drug produced such a toxic reaction.

Because of the rather widespread clinical use of nitrogen mustard in the treatment especially of neoplastic diseases of the hemopoietic system and the serious implication of hemorrhage, studies in experimental animals were undertaken to elucidate further this effect.

MATERIALS AND METHODS

Swift's snuffle-free rabbits of uniform age and weight (2.5 to 3 kilograms) were used in this study. Previous studies with nitrogen mustard in our laboratories[9] indicated that a single intravenous dose of 3.0 mg. per kilogram of body weight produced a destructive effect on the hemopoietic system of this strain of rabbits approximately equivalent to 800 r total-body X irradiation. The effect of both of these agents on the whole-blood coagulation was studied simultaneously for comparative purposes.

Technique of Nitrogen Mustard Administration.—A dose of 3 mg. of nitrogen mustard per kilogram of body weight was injected slowly into the marginal ear vein of the rabbit immediately after the chemical had been dissolved in physiologic saline. A concentration of 1 mg. of the drug per 1 c.c. of saline was used since this concentration produced no sloughing of the ear tissue and did not obliterate the ear vein.

Technique of X Irradiation.—The x-rays administered in these experiments were generated on a 200 kv. machine operating at 15 milliamperes. A 0.5 mm. copper filter and a 1 mm. aluminum filter were used. The half-value layer in copper of the filtered beam was 0.98 millimeter. The exposure was measured with a Victoreen condenser r-meter equipped with a 100 r chamber. Measurements were made in air within a treatment box at the position occupied by the center of the animal's body. Victoreen chambers of 250 r full scale readings were used as monitors.

Hematologic Studies.—

Whole-blood Clotting Time: The clotting time of the whole blood was determined at room temperature by a modification of the Lee-White method.[10] Whole blood (0.5 c.c. amounts) was measured into five or six small test tubes (10 by 75 mm.) and 1 c.c. amounts were pipetted into two or three large test tubes (13 by 100 mm.). The larger tubes were not examined until clotting had been established in the small tubes. The time which elapsed between obtaining the blood (first appearance in the syringe) by cardiocentesis and the clotting of two consecutive tubes which had not previously been manipulated was considered to be the whole-blood clotting time, and the blood was considered to be clotted when it remained firm upon inversion of the tube.

Platelet Determination.—Platelet counts were made by drawing blood to the 0.5 mark in a white blood diluting pipette and diluting to the 11 mark with a 1.8 per cent solution of freshly prepared sodium citrate. The pipette was gently shaken for one minute after which the entire content of the pipette was allowed to flow into a small Lusteroid conical-shaped tube. The tube was set aside at room temperature until sedimentation of the red cells had occurred. The platelet counts were then made on the plasma whenever time permitted (within twenty-four hours).

TABLE I. AMOUNTS OF PROTAMINE SULFATE AND BLOOD USED IN THE HEPARIN TOLERANCE TEST

TUBE	1	2	3	4	5	6	7	8	9	10
% Protamine sulfate	5.0	10.0	15.0	17.5	20.0	22.5	25.0	27.5	30.0	35.0
Heparinized blood* (c.c.)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Amount of protamine sulfate† (c.c.)	0.01	0.02	0.03	0.035	0.04	0.045	0.05	0.055	0.06	0.07

*0.02 c.c. liquid heparin in 3.5 c.c. blood.
†1 mg. protamine sulfate in 2 c.c. normal saline.

Heparin Tolerance Test.—Modifications of the heparin tolerance technique developed by Allen and co-workers[4] were necessary to avoid the repeated withdrawal of large amounts of blood from rabbits. Protamin sulfate which was weighed on an analytical balance was dissolved in physiologic saline in a concentration of 1 mg. of protamine to 2 c.c. of saline. This solution, which was prepared every other day, was titrated into a series of ten small test tubes (10 by 75 mm.) as shown in Table I. In performing the test, 3.5 c.c. of blood obtained by cardiocentesis were immediately added to a tube containing exactly 0.02 c.c. of liquid heparin and gently inverted twenty times. Immediately thereafter, 0.25 c.c. of this heparinized blood was added to tubes containing the graduated amounts of protamine sulfate described. Each tube then was shaken gently and set aside at room temperature. At the end of one, three, and twenty-four hours, readings were made to determine the minimum amount of protamine sulfate necessary to cause the blood to clot. Normal rabbit blood requires 17.5 to 22.5 γ protamine to bind the heparin in the amounts used. With one exception, the one-hour readings are reported in this communication.

Prothrombin Studies.—Prothrombin determinations were made according to the Link-Shapiro modification of Quick's method.[11] Commercial thromboplastin (Maltine) and both diluted and undiluted blood plasma were used in these determinations.

Calcium and Fibrinogen.—Calcium determinations were made on the blood serum by precipitating the calcium as an oxalate according to the method of Clark and Collip.[12] The presence of fibrinogen was noted by heating the plasma to 58° C. in a water bath. No quantitative fibrinogen determinations were made.

GENERAL PROCEDURE

Rabbits were divided into three groups for the nitrogen mustard experiments. Group I received nitrogen mustard (3 or 4 mg. per kilogram of body weight) intravenously. Group II received 3 or 4 c.c. of physiologic saline per kilogram of body weight intravenously. Group III consisted of normal untreated animals that received neither nitrogen mustard nor saline. Since saline was used as a solvent for the nitrogen mustard, Group II animals that received saline served as a control for Group I, while Group III which received neither nitrogen mustard nor saline served as a control for both Groups I and II.

Approximately 10 c.c. of blood were withdrawn from the rabbit by cardiocentesis using a dry 18 gauge needle and a dry 10 c.c. syringe. Three and one-half cubic centimeters of this blood were immediately transferred to a graduated centrifuge tube containing 0.02 c.c. of liquid heparin for the heparin tolerance test; 0.9 c.c. of blood was transferred to a tube containing 0.1 c.c. of sodium oxalate for the prothrombin determination; the remaining blood was pipetted in 0.5 and 1 c.c. amounts into a series of test tubes for the whole-blood clotting time as previously described.

Only one attempt was made to obtain blood from the heart of the rabbit. When that was unsuccessful no further attempts were made to obtain blood until the animal had rested (at least two hours).

After a prolonged clotting time and an abnormal heparin tolerance test had been established, one group of animals was given toluidine blue (64 per cent dye content) intravenously, while another group was treated with protamine sulfate. Both toluidine blue and protamine sulfate were given in concentrations of 1 mg. per 1 c.c. saline. The doses of protamine or toluidine blue given per injection were 3 and 4, and 2 and 2.5 mg., respectively, per kilo-

gram of body weight. One group of animals that received 3 mg. of nitrogen mustard per kilogram of body weight remained untreated for comparative purposes.

Animals exposed to 800 r total-body X radiation were treated similarly, one group receiving either toluidine blue or protamine after a prolonged clotting time had been established, while another group remained untreated.

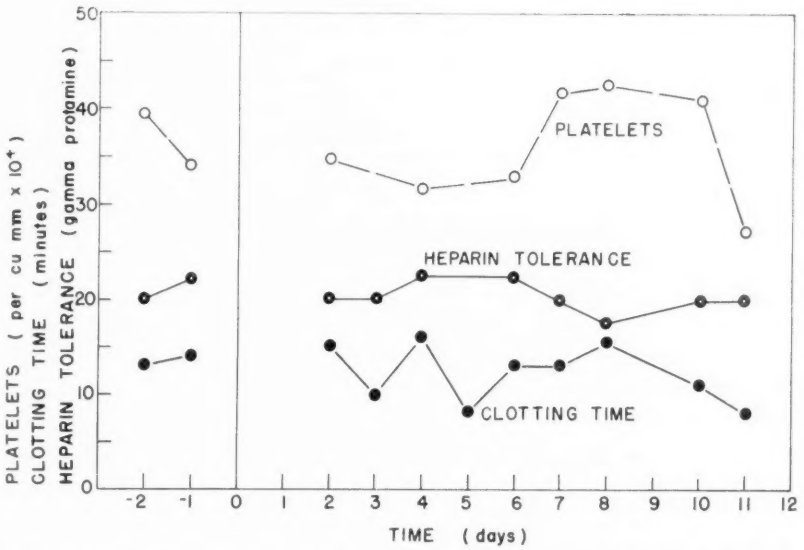
PLATELETS (per cu mm x 10⁴)
CLOTTING TIME (minutes)
HEPARIN TOLERANCE (gamma protamine)
PLATELETS
HEPARIN TOLERANCE
CLOTTING TIME
TIME (days)

Fig. 1A.—Average clotting time (1 c.c. blood), platelet values, and heparin tolerance test (one-hour results) in normal rabbits (blood obtained by cardiocentesis).

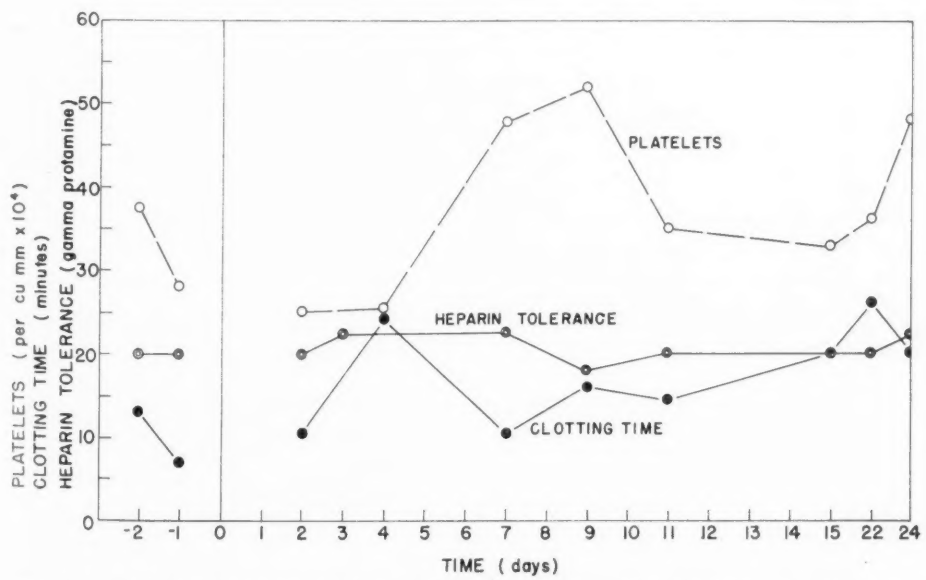
PLATELETS (per cu mm x 10⁴)
CLOTTING TIME (minutes)
HEPARIN TOLERANCE (gamma protamine)
PLATELETS
HEPARIN TOLERANCE
CLOTTING TIME
TIME (days)

Fig. 1B.—Clotting time (1 c.c. blood), platelet values, and heparin tolerance test (one-hour results) in one normal control rabbit (0785) (blood obtained by cardiocentesis).

RESULTS

The mean average of the whole-blood clotting time in normal untreated rabbits varied from 8 to 16 minutes in tubes containing 1 c.c. of whole blood. The platelets of the normal rabbits varied considerably but were usually between 250,000 to 500,000 per cubic millimeter. The heparin tolerance test gave fairly constant results in normal rabbits; 17.5 to 22.5 γ of protamine sulfate were necessary to produce a clot in heparinized blood prepared as outlined. Thus clotting occurred normally in tube 4, 5, or 6 (see Table I). The mean average results of the whole-blood clotting time, platelets per cubic millimeter, and the heparin tolerance results of seven untreated normal animals are shown in Figs. 1*A* and 1*B*. Fig. 1*A* illustrates the fluctuations in these determinations which a single animal may exhibit during a twenty-four day period of observation. Normal animals on which such determinations are made at frequent intervals over a period of thirty days or more usually exhibit less variation than this individual animal illustrates.

Effect of Nitrogen Mustard.—

Clotting Time: A prolonged clotting time of the whole blood occurred after the intravenous injections of 3 or 4 mg. of nitrogen mustard per kilogram of body weight. Fig. 2 shows the effect on the clotting time of sixteen rabbits given intravenous doses of 3 mg. per kilogram. An increase in the length of the clotting time was present twenty-four hours after nitrogen mustard administration, reached a peak in *circa* four to seven days, remained high about ten days, and returned to a normal value in the surviving animals by two weeks. The effect of nitrogen mustard on the clotting time of an individual animal is shown in Fig. 3. The pattern of individual response is similar to the mean values shown in Fig. 2 except that the clotting time was not infrequently prolonged to 50 and 60 minutes in individual animals.

Platelets.—The data from sixteen animals studied consecutively after nitrogen mustard administration are shown in Fig. 2. The mean maximum decrease in the platelets per cubic millimeter had occurred by the fourth day after nitrogen mustard administration, and remained below normal values for *circa* seven days. The lowest mean platelet level (150,000 per cubic millimeter) did not parallel the high peak in the clotting time on the seventh day, but the return of the clotting time to normal limits paralleled the return of the platelets to normal values. Even more striking is the fact that the clotting time in practically all animals had increased prior to a significant reduction in platelet values. These facts are clearly illustrated in individual animals as is shown in Fig. 3.

Heparin Tolerance.—In general, the effect of nitrogen mustard on the heparin tolerance paralleled the effect on the clotting time. As the clotting time of the animal rose, a greater concentration of protamine sulfate was necessary to produce a clot in the heparin tolerance tubes in one hour. This is illustrated in Figs. 3 and 6.

Fibrinogen and Calcium.—Without exception fibrinogen was present in all plasma samples. The blood calcium determinations of the nitrogen mustard

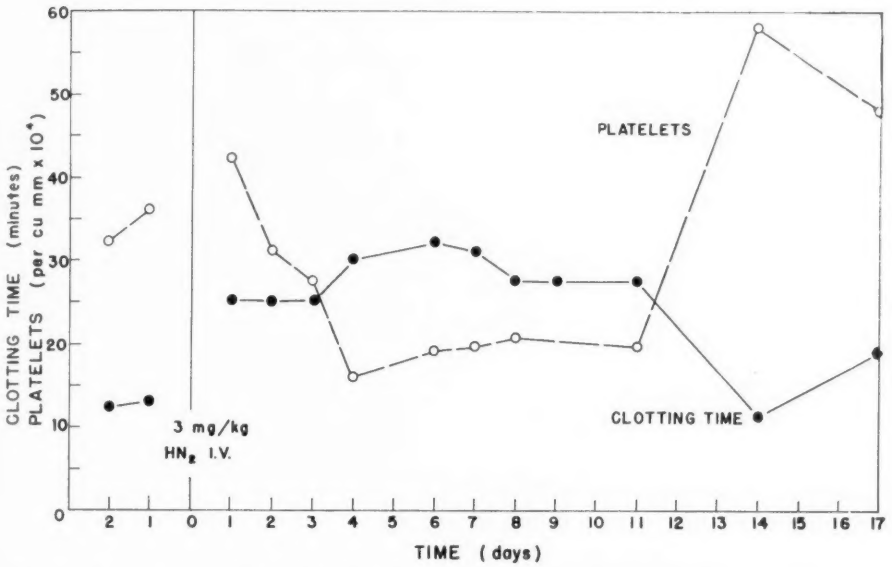

Fig. 2.—Clotting time (1 c.c. blood) and platelet values in sixteen rabbits following the intravenous administration of 3 mg. per kilogram of nitrogen mustard.

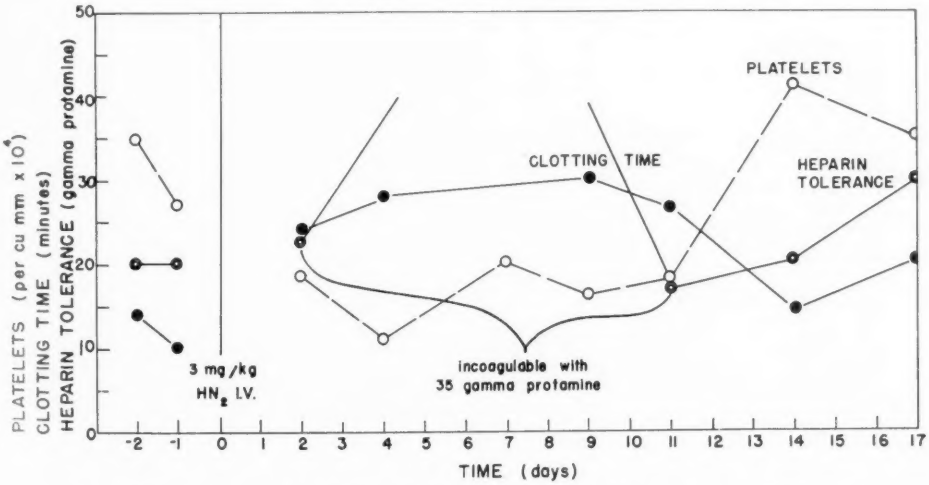

Fig. 3.—Clotting time (1 c.c. blood), platelet values, and heparin tolerance test (one-hour readings) in one rabbit (0733) following the administration of 3 mg. per kilogram nitrogen mustard.

treated animals with delayed clotting and an abnormal heparin tolerance test were within the normal range (13 to 14 mg. per cent).

Prothrombin Time.—As stated, blood for a prothrombin time determination was obtained simultaneously with blood for clotting time and heparin tolerance determinations in all control and mustard-injected animals. In no instance was a prolonged prothrombin time observed in animals which had a prolonged clotting time or abnormal heparin tolerance test as a result of the nitrogen mustard intoxication.

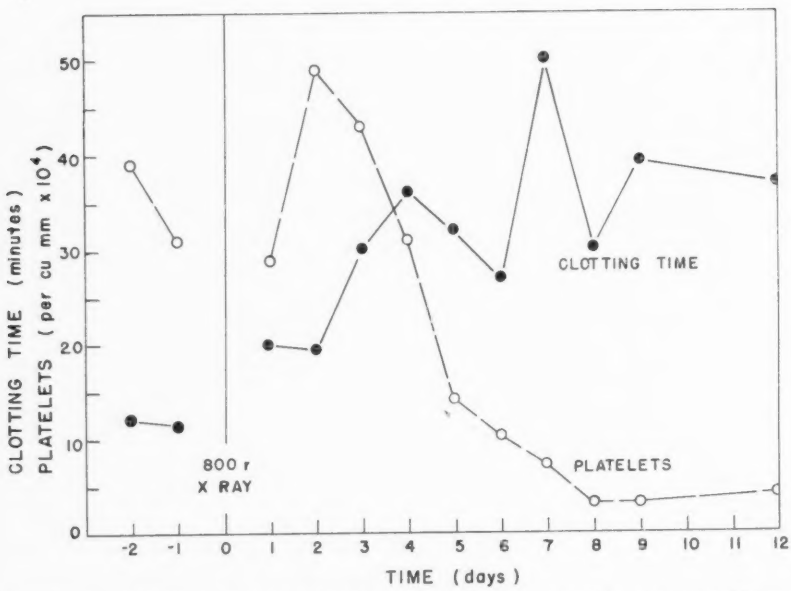

Fig. 4.—Average clotting time (1 c.c. blood) and platelet values in sixteen rabbits after 800 r total-body X irradiation.

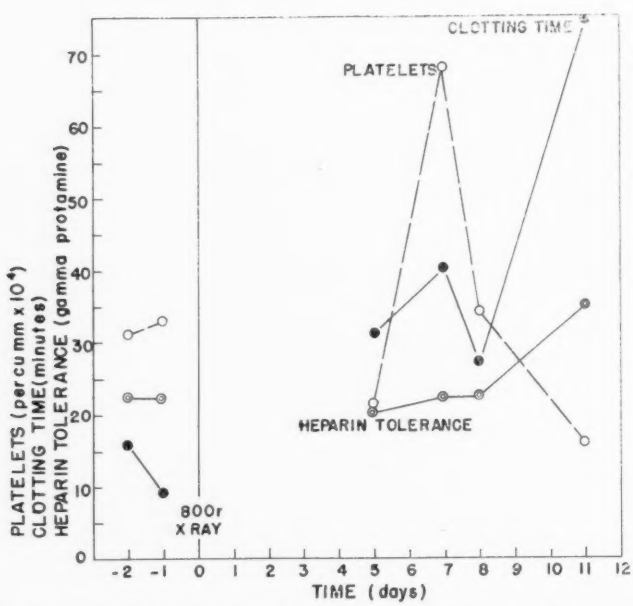

Fig. 5.—Clotting time (1 c.c. blood), platelet values, and heparin tolerance test (one-hour results) in one rabbit (0762) after 800 r total-body X irradiation.

Effect of X-Rays.—Exposure to 800 r total-body X radiation produced results similar to those described following nitrogen mustard administration except that the prolonged clotting time persisted for a longer period of time and the platelets, which were reduced by the fifth day, remained reduced for a period of seven days or longer. Fig. 4 is representative of this group

of sixteen animals. The heparin tolerance test, clotting time and platelet values of one animal following irradiation are shown in Fig. 5. These results are representative of those animals which did not survive.

Prothrombin time remained unchanged in the animals exposed to 800 r X radiation. The blood calcium values likewise remained within normal limits and fibrinogen was present in all plasma samples.

Effect of Antiheparin Substances on the Prolonged Clotting Induced by Nitrogen Mustard or X-Ray.—The effect of intravenously administered toluidine blue or protamine sulfate on the clotting time of normal animals and animals with a nitrogen mustard-induced prolongation in the clotting time

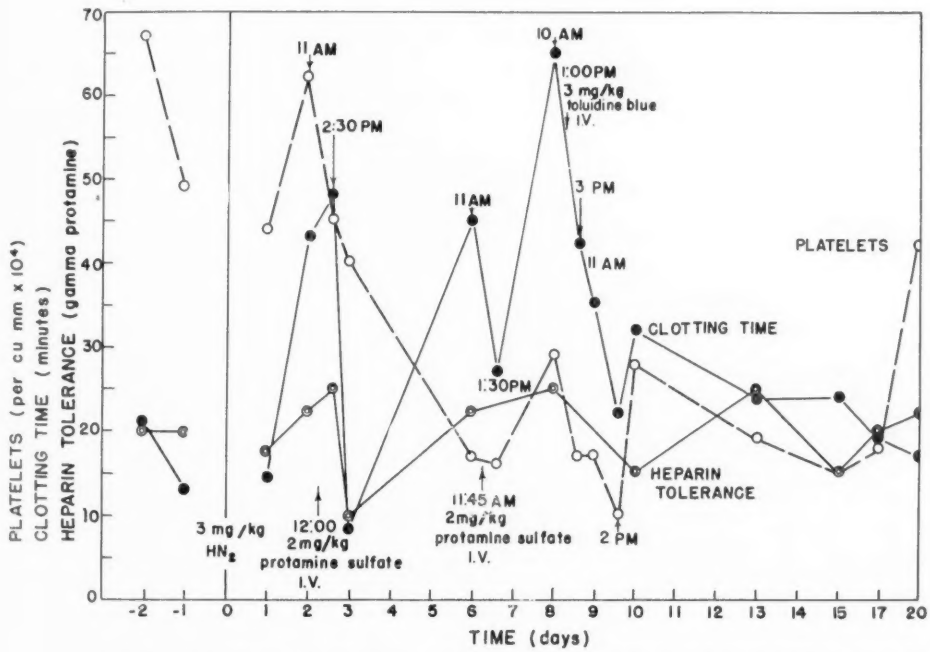

Fig. 6.—The effect of antiheparin substances (toluidine blue and protamine sulfate) on the prolonged clotting time (1 c.c. blood), platelet values, and decreased heparin tolerance (three-hour results) in a rabbit (0718) produced by the intravenous administration of 3 mg. per kilogram of nitrogen mustard.

was studied repeatedly in individual animals. Single doses of from 3 to 4 mg. per kilogram of toluidine blue were administered either intracardially or intravenously (ear vein), and observations on the clotting time, heparin tolerance, and platelet values were made at two and twenty-four hours after injection. The same procedure with a dosage range of 2 to 3 mg. per kilogram was followed when the effect of protamine sulfate was similarly investigated.

The effect of intravenous injections of toluidine blue and protamine sulfate on the whole-blood clotting time and heparin tolerance test of an individual rabbit following 3 mg. per kilogram of nitrogen mustard is shown in Fig. 6. The intravenous injection of 2 mg. of protamine reduced the clotting time from 48 to 8 minutes in two hours. The amount of protamine neces-

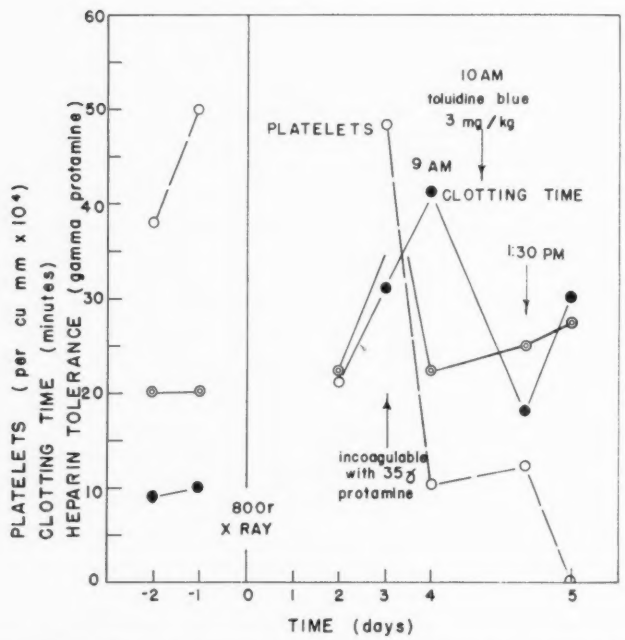

Fig. 7A.—The effect of toluidine blue on the prolonged clotting time (1 c.c. blood), platelet count, and heparin tolerance (one-hour readings) in a rabbit (0780) following 800 r total-body X irradiation.

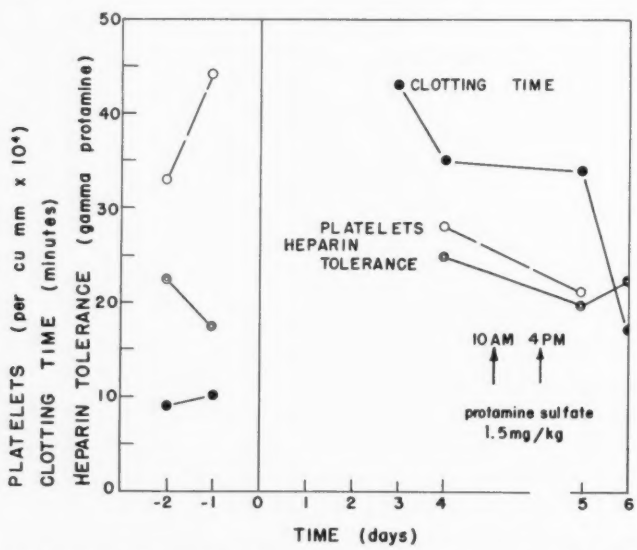

Fig. 7B.—Effect of repeated doses of protamine sulfate on the prolonged clotting time, platelet values, and heparin tolerance in a rabbit (0764) following 800 r total-body X irradiation.

sary to produce a clot in the heparin tolerance test was reduced from 25 to 10 γ during the same period. The intravenous injection of 3 mg. of toluidine blue per kilogram of body weight gave similar results in the same rabbit six days after the first injection of protamine when the clotting time was again prolonged. After the toluidine blue injection, the clotting time dropped from 65 minutes to 42 minutes in two hours, and then to 35 minutes in twenty-four hours. Another injection of 4 mg. of toluidine blue reduced the clotting time to 22 minutes in two hours, after which the animal made an uneventful recovery.

No significant change was noted in the number of platelets per cubic millimeter following the injections of toluidine blue or protamine sulfate.

Without exception in the presence of a nitrogen mustard-induced prolongation in the clotting time, the intravenous injection of toluidine blue in 3 or 4 mg. per kilogram doses or of protamine sulfate in doses ranging from 1.5 to 2.5 mg. per kilogram significantly reduced the clotting time. In some instances the clotting time was reduced to the normal range by two hours, or significant reduction in the clotting time was produced by two hours but was not reduced to the normal range. In others, the clotting time was significantly reduced by two hours, reached the normal range twenty-four hours after injection of toluidine blue, but by forty-eight hours was again prolonged.

In rabbits exposed to 800 r total-body X radiation, the effects of toluidine blue and protamine sulfate on the prolonged clotting time are shown in Figs. 7*A* and 7*B*. Although the clotting time was reduced in rabbit number 0780 (Fig. 7*A*) from 41 minutes to 18 minutes in two hours after the intravenous administration of 3 mg. of toluidine blue per kilogram of body weight, the effects on the heparin tolerance test were not remarkable. The following day the clotting time was again prolonged (30 minutes), and an increased amount of protamine was necessary to bind the heparin in the heparin tolerance test. Before another injection of toluidine blue could be administered, the animal died. Occasionally, as in this animal, an abnormal heparin tolerance fails to revert to normal two hours after the intravenous administration of an antiheparin substance even though the clotting time was returned to normal or significantly reduced. By twenty-four hours after toluidine blue or protamine injection however, the heparin tolerance was normal if the clotting time had returned to normal.

Animal number 0764 received protamin sulfate in 1.5 mg. per kilogram amounts intravenously at 10 A.M. and 4 P.M. when the clotting time was 43 minutes and when 35 γ of protamine did not clot the blood in the heparin tolerance test. The following day when the clotting time was 34 minutes, another injection of protamine (1 mg. per kilogram) was given intravenously. Although the clotting time was reduced to 17 minutes in twenty-four hours after the last injection of the protamine sulfate, the animal did not survive. It is likely that these animals died as a result of the cardiocentesis or from the general X irradiation effects rather than from the protamine or toluidine blue injections.

DISCUSSION

A prolonged clotting time and an abnormal "heparin tolerance" were almost invariably produced in rabbits given a single intravenous dose of 3 mg. per kilogram or more of nitrogen mustard (methyl-*bis*-(β-chloroethyl) amine hydrochloride). A similar effect was produced in rabbits after exposure to 800 r whole-body X radiation. The prolonged clotting time appeared by twenty-four hours in rabbits given nitrogen mustard, whereas significant prolongation in clotting time was not consistently observed until the second or third day after irradiation. Spontaneous recovery from this effect occurred earlier in the nitrogen mustard treated animals than in irradiated animals. The effects of a single injection (3.0 mg. per kilogram) of nitrogen mustard on the cellular elements and hemoglobin of the peripheral blood and the histologic effect on the hemopoietic tissues are essentially comparable with those produced by 800 r whole-body X irradiation. Recovery of the bone marrow and lymphatic tissue and return of the cellular constituents of the peripheral blood to normal values after doses of these two agents is more rapid in nitrogen mustard treated animals than in the irradiated animals.[9] Since, however, these two agents are not strictly comparable in terms of selectivity and mechanism of action, one cannot expect the appearance of or recovery from largely identical biologic effects to be the same.

The mechanism of the prolonged clotting time and altered heparin tolerance, which appears after nitrogen mustard intoxication, is probably identical with that described by Allen and Jacobson[2] in the dog after exposure to lethal amounts of X radiation, namely an increase in a circulating heparin or heparin-like substance. This is substantiated by the facts that the prothrombin time, fibrinogen, and calcium levels were normal in the animals with a prolonged clotting time; no fibrinolysin was demonstrated; and antiheparin substances, such as toluidine blue and protamine, were capable of reversing to normal values or significantly reducing the prolonged clotting time for varying lengths of time. The recent report by Smith, Jacobson, and co-workers[5] has shown that therapeutic doses of nitrogen mustard produced this same effect in the human being. It is of interest in this connection that the dose required to produce this clotting defect in the rabbit with regularity is about seven times the total dose usually used for therapeutic purposes in the human being.

In the original publication by Allen and Jacobson[2] it was pointed out that not infrequently a prolonged clotting time was observed in X-irradiated dogs before thrombocytopenia developed. In the rabbit, after intoxication with either nitrogen mustard or x-ray, the clotting time is almost always significantly increased prior to significant reduction in platelet values. On the other hand, the maximum increase in clotting time roughly parallels or occurs concomitantly with the maximum platelet decrease. Spontaneous recovery of both to normal levels occurs at practically the same time. Reversal of the clotting time and heparin tolerance to the normal range by the intravenous administration of toluidine blue or protamine sulfate did not significantly

alter the platelet level. These facts do not necessarily indicate, however, that platelets have no role in the production of this "syndrome."

The results of these experiments are interesting from the standpoint that a clotting defect is produced by nitrogen mustard which is similar to that produced by ionizing radiations. It seems likely that other chemical substances with the capacity to produce severe damage to the blood-forming tissue may likewise produce a prolonged clotting time in which heparin or a heparin-like substance is involved. It is an important fact that spontaneous recovery from this hemorrhagic syndrome produced by either nitrogen mustard or irradiation is possible. It is also significant that antiheparin substances are therapeutically effective in the treatment of this syndrome.

SUMMARY AND CONCLUSIONS

A prolonged clotting time is produced in rabbits by the intravenous administration of 3 or 4 mg. of nitrogen mustard per kilogram of body weight. The same syndrome is produced in human beings after therapeutic doses of this drug. The amount of protamine necessary to produce clotting in the heparin tolerance test is increased in those rabbits that develop a prolonged clotting time.

The prolonged clotting time occurs in rabbits after a 3 mg. per kilogram dose within twenty-four hours after administration and persists for approximately twelve days. Spontaneous recovery occurs in surviving animals by the twelfth to fourteenth day. A maximum reduction in blood platelets occurs by the fourth day in rabbits receiving 3 mg. per kilogram nitrogen mustard, with recovery by the twelfth to fourteenth day. Thus, the clotting time is prolonged prior to significant platelet reduction. However, the period of platelet reduction (fourth through twelfth day after nitrogen mustard administration) roughly parallels the period in which maximum increase in the clotting time exists. Spontaneous recovery of the clotting defect and the return of platelet values to normal levels in the peripheral blood occur at a comparable time (*circa* fourteen days).

Animals which developed a prolonged clotting time had normal calcium and prothrombin values.*

The prolonged clotting time and decreased "heparin tolerance" are reversible with antiheparin substances. The anticoagulant present in the blood is probably heparin or a heparin-like substance.

The clotting defect produced by nitrogen mustard resembles that produced by whole-body X irradiation (800 r) in rabbits and is also reversible with antiheparin substances.

It is suggested that the potentially serious clotting defect may be produced by other agents producing toxic effects directly or indirectly on the blood-forming tissue. This should be borne in mind since a method of treatment is available (antiheparin substances) which may make it possible to pass the critical period of potential hemorrhage until spontaneous recovery ensues.

*Since submission of the manuscript, quantitative fibrinogen determinations have been done on a few animals in which a prolonged clotting time was present after nitrogen mustard administration. In these animals, the fibrinogen values were normal.

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THE USE OF PLASMODIUM VIVAX PRESERVED BY FREEZING IN INDUCING MALARIA

GEORGE M. SAUNDERS, M.D., DAVID W. TALMAGE, M.D., AND VIRGIL SCOTT, M.D.
ST. LOUIS, MO.

FEVER from induced malaria continues to be widely employed in the management of neurosyphilis, particularly in parenchymatous forms such as dementia paralytica and primary optic atrophy. A serious obstacle to the use of malaria has been the difficulty of maintaining a source of parasites. Even in metropolitan areas, cases of malaria naturally acquired or deliberately induced are frequently not available. This difficulty has increased since the introduction into syphilotherapy of penicillin. Milder forms of neurosyphilis are now treated effectively with this antibiotic alone; consequently, treatment with induced fever is less frequently necessary and fewer cases of malaria are to be found. It may be troublesome and time consuming to obtain malarial blood from distant medical centers, and unless shipment is rapid and delivery prompt, the blood may be noninfectious by the time inoculation is accomplished. Since malarial parasites remain viable at room temperature for three to five days at most, a simple, effective method for their preservation for longer periods has practical value.

By a method of quick freezing of malarial blood and storage at low temperatures, we have had available a supply of *Plasmodium vivax* parasites which has been used successfully during the past year to induce malaria in approximately forty patients in hospitals in the St. Louis area.

A preliminary report[1] briefly outlined the method used and cited successful inoculations in six patients, three of which were described in detail. It is the purpose of the present paper to record the experience with a larger series of cases.

It is well known that many infectious agents, bacterial and viral, can be maintained in the frozen state. As summarized in our previous communication, several types of pathogenic protozoa, including those of avian and monkey malaria, have been similarly preserved.[1] The only previously recorded attempt at preserving human plasmodia was that of Coggeshall[2] who was unsuccessful using a method which involved rapid freezing at –72° to –80° C. followed by slow thawing. The procedure employed in the present study is fundamentally similar except that thawing has been effected rapidly rather than slowly.

ORIGIN OF PARENT STRAINS

H. K. Strain.—This strain was isolated April 8, 1947, from a 29-year-old veteran during an acute episode of relapsing malaria. H. K. had been exposed in the Southwest Pacific area, New Guinea, and the Philippines, from June, 1944, to December, 1945. Throughout this time, while on routine quinacrine suppression, no clinical attacks of malaria occurred.

From the Departments of Preventive Medicine and Internal Medicine, Washington University, and the Syphilis Clinic of the Washington University Clinics.

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Suppressive therapy was stopped in December, 1945, when he returned to the United States; a few weeks later he suffered his first acute attack of malaria which was followed by repeated relapses, about ten in all, between February, 1946, and April, 1948. Blood first was drawn for preservation on April 8, 1947, after three paroxysms, the last one having occurred four to six hours before venipuncture. Blood films showed numerous amoeboid and younger forms of *P. vivax* with a parasite density of about 10,000 per mm.^{3} During the course of the next year, partly through oversight and the more frequent use of another preserved strain of *P. vivax*, the H. K. strain was lost, but was recovered in April, 1948, when H. K. again suffered a relapse. Blood drawn a few hours after a shaking chill when the patient's temperature was 39.2° C. showed many young ring forms, about 20,000 per mm.^{3}

N. Y. Strain.—This strain was isolated June 13, 1947, from a 29-year-old war veteran on the first day of a relapse. While serving in the Philippines during 1944-1945, N. Y. suffered his initial attack of malaria. Since discharge from the Army he had been having relapses every two to three months. At the time of blood withdrawal numerous young ring forms of *P. vivax* were present on smear; a parasite count indicated about 5,000 per mm.^{3}

METHOD OF PRESERVATION*

Blood from malarial donors was prevented from clotting by the use of sodium citrate or by defibrination with glass beads. Citrate was employed in a 4 per cent solution in a ratio of 5 parts of blood to 1 part of sodium citrate, giving a final concentration of less than 1 per cent of the salt. A part of the citrate was drawn into the collecting syringe in order to eliminate possible coagulation in the syringe; then the required amount of blood was drawn and the contents of the syringe were added to a flask of 125 ml. capacity containing the remainder of the citrate. Defibrination was accomplished by adding the blood to a flask containing a layer of glass beads and shaking or rotating the contents gently until the fibrin collected in a mass about the beads.

After citration or defibrination the blood was transferred in 2 to 4 ml. amounts to thin-walled glass ampules, about 15 by 80 mm. in size, and the ampules were sealed by flaming. The ampules were then immersed in a previously prepared freezing solution consisting of ethyl alcohol and dry ice at a temperature of −70° to −80° C. Ampules were immersed individually while being rotated rapidly in order to disperse the contained citrated blood into a thin film on the inner surface of the ampule. Freezing was thus accomplished in the course of a few seconds. After remaining in the freezing mixture approximately ten minutes, the ampules were transferred to a dry-ice box for storage at −70° C. A commercial home freezer with a specially constructed insulated inner cabinet containing a central space for storage of ampules and compartments at the ends for dry ice has proved satisfactory for this purpose. The entire process, from venipuncture to storage, was completed as rapidly as possible, usually requiring no longer than thirty to forty-five minutes. Aseptic precautions were observed throughout.

Ampules were removed as needed, rapidly thawed at the bedside by immersion in a water bath at 40° C., and the fluid immediately was injected intravenously into the recipient. The usual inoculum consisted of the contents of two or three ampules, a volume of 4 to 10 ml., although as little as 2 ml. has been used successfully.

TRANSFER OF STRAINS

Following the development of clinical malaria, patients were bled after varying numbers of paroxysms and during various phases of the life cycle of the parasite, and the freezing and storage process was repeated. Thus, the parent strains were maintained by serial passage from one patient to another. Inoculations were performed uniformly with material which had been frozen and resort to direct transfer was not necessary.

*Although the method described includes minor modifications adopted during the course of the study, the procedure has remained essentially unchanged.

DESCRIPTION OF SUBJECTS

Since the beginning of the experiment a little more than a year ago a total of fifty-four subjects has been inoculated with preserved *P. vivax* blood, one subject being inoculated twice, for a total of fifty-five inoculations. All subjects were patients with neurosyphilis in whom malaria was being induced for therapeutic purposes. All were adults; forty-eight were men and six were women; all were white except one Negro and one Mexican of mixed blood. The racial selection was premeditated since it is well known that Negroes are frequently immune to vivax malaria. The patients were hospitalized in ten different hospitals in the St. Louis area.

RESULTS

Results of inoculation have been classed as successes, failures, incomplete data, and lost to observation, according to the following criteria. An inoculation was considered a success when it was followed by oral temperatures of 37.8° C. or more on at least one occasion forty-eight hours or more following inoculation in addition to the demonstration of malarial parasites in thick or thin blood preparations. In successful cases the incubation period was calculated as the time in days from inoculation to the first febrile elevation.

Cases were officially classed as failures when parasites were not found in the blood during the period from about six days to four or five weeks after inoculation. However, two patients, who had irregular low-grade fever on the tenth to fourteenth postinoculation days without parasites being demonstrated, subsequently developed typical malarial paroxysms with demonstrable parasites, one forty-five days and the other seventy-eight days after inoculation. Hence it seems likely that some of the patients who were classed as failures after four to six weeks and who were then lost from observation or given antimalarial therapy might have developed malaria. Cases classed as incomplete include those patients whose postinoculation period is currently less than four weeks without clinical or laboratory evidence of malaria and two patients who were discharged from hospitals less than three weeks after inoculation, who developed chills and fever at home, recorded temperatures exceeding 39° C., and who promptly became afebrile following antimalarial therapy, blood smears not being available for examination during the posthospital period. In the two cases lost to observation the patients left the hospital less than a week following inoculation and subsequent observations are lacking.

Of the fifty-five inoculations there are at present forty-six cases with data adequate to classify as success or failures. Table I shows that forty, or 87 per cent of the forty-six, developed malaria, and six, or 13 per cent, were classed as failures. These six failed to develop malaria clinically and parasites were never demonstrated by smear over observation periods ranging from thirty-four to ninety days.

TABLE I. RESULTS OF INOCULATION OF P. VIVAX STRAINS PRESERVED BY FREEZING

RESULT	STRAIN N. Y.	STRAIN H. K.	TOTAL
Successes	34	6	40
Failures	4	2	6
Total	38	8	46
Incomplete	1	5	7
Lost	2	0	2
Total	41	13	55

Sufficient information relative to possible strain differences has not been accumulated thus far. Among the cases for which we have adequate data, of thirty-eight inoculations performed with N. Y. strain, thirty, or 90 per cent, have been successful; six, or 75 per cent, of eight inoculated with H. K. strain developed malaria.

In considering causes of failure other than technical error, three factors deserve mention—previous malarial infection, race, and the inadvertent suppression of the malaria by drugs possessing antimalarial activity. Whether any of the six known failures had suffered previously from naturally acquired malaria and might, therefore, have been immune is not definitely known. Two of these patients, so demented that historical information on this point could not be obtained, had lived in malarious regions. One was a Greek who had lived in his native country for an eighteen-year period; the other was a Mexican who was also probably part Negro. A third patient, classified as a failure after forty-five days of observation following an initial inoculation with the H. K. strain, subsequently was reinoculated successfully with the N. Y. strain, the incubation period (sixteen days) being within the usual range. In this patient the original inoculation was probably faulty since it seems less likely that strain differences would have influenced the result. Failure may be explained in the case of the one Negro subject on the basis of race resistance.

None of the forty who developed malaria are known to have had the naturally acquired disease, but one had been previously inoculated (unsuccessfully) with fresh blood containing quartan parasites.

In reference to antimalarial drugs, it is well known, although perhaps not widely recognized, that both the sulfonamides and the heavy metal antisyphilitic agents,* including the arsenicals and bismuth, may abort or suppress infection with malarial parasites. The same effect may be produced by even minute doses of quinine comparable to those contained in some of the commonly used "tonics." To our knowledge, none of the failures were related to the administration of these drugs, although several of these patients were not under our own observation. It may be of interest to refer to one patient who developed severe convulsive seizures a few days after inoculation and a day or two after penicillin therapy was instituted. To control the convulsions the patient was given regular doses of phenobarbital, and Dilantin sodium, 90 mg. three times a day. It was felt that his failure to develop malaria might have been due to some suppressive action of the barbiturates or Dilantin since it is known that many types of complex organic compounds may act as antimalarials. To test the supposition that Dilantin might act in this manner, one patient in whom it was desired to terminate malaria was given Dilantin sodium, 90 mg. three times a day for a period of four days. He suffered a regular malarial paroxysm on the first day of Dilantin treatment but was afebrile subsequently for five days, during which malaria parasites were not found in blood smears. At this time the patient was given a course of chloroquine to make sure the malarial infection was terminated. In another patient, similar doses of Dilantin for three days failed to have any apparent effect upon the malaria, and because of his critical condition the patient was treated with chloroquine to end the attack.

*But not penicillin.

In attempting to analyze the results of inoculation there are a number of variable factors, some of which may be controlled and measured with reasonable accuracy, while others are beyond control and cannot be measured with present methods. The measurable, controllable factors include parasite strains, the number of parasites in the inoculum before freezing, the stage of development of the parasites, the method of preventing clotting, the speed of freezing and thawing, the storage temperatures, and the duration of preservation. The uncontrollable, incalculable factors include the host resistance whether due to previous malaria or other factors including race.

Parasite Strains.—The differences in results of inoculations with the two strains used in this study including the proportion of successes to failures, and the incubation period in the successes, are too small to be of significance.

The Size of Inoculum.—It might logically be assumed that few parasites might fail to produce malaria, or produce it only after a prolonged incubation period, and that a large number of parasites would produce malaria more consistently and with a relatively short incubation period. In most instances a rough parasite count was made on donor's blood by relating the number of parasites found in stained smears to the number of leucocytes seen and to the total leucocyte count. But, although we can thus estimate the number of parasites in a given quantity of blood before freezing, it has not been possible to determine how many survive the freezing, thawing, and storing.

There is no evidence that the total number of parasites in the inoculum before freezing accounted for success or failure in producing malaria in the host. In the six cases classed as failures the number of parasites was greater on the average than in the cases classed as successes, and the blood had been preserved for shorter periods of time. Furthermore, when the successes are grouped according to number of parasites in the inoculum, as shown in Table II, there is no evidence that the incubation period was altered significantly by increasing the number of parasites. The length of time the parasites were stored was essentially comparable in all groups except the fourth, in which there was a much longer average storage time and a slightly shorter incubation period.

TABLE II. THE SIZE OF INOCULUM, THE LENGTH OF STORAGE OF PARASITES, AND THE INCUBATION PERIOD IN CASES OF SUCCESSFUL INOCULATION

SIZE OF INOCULUM; NUMBER OF PARASITES IN MILLIONS (PER MM.^{3})	TIME FROZEN IN DAYS		INCUBATION PERIOD IN DAYS		NUMBER OF CASES
	MEAN	EXTREMES	MEAN	EXTREMES	
9-19	31.7	10-102	12.8	9-14	7
21-40	31.1	6-85	12.6	10-22	14
41-69	54.4	6-166	11.3	7-16	12
100-137	26.5	3-59	15.0	12-20	4

The Stage of Development of Parasites.—It is possible that the resistance of parasites to freezing and thawing and prolonged storage at low temperatures might vary with their age, size, and complexity of structure, that is, with the stage of development. As a working hypothesis it was assumed that the merozoites, or very young trophozoites, might be the most resistant, and the mature

TABLE III. THE STAGE OF DEVELOPMENT OF PARASITES RELATED TO THE INCUBATION PERIOD

STAGE OF DEVELOPMENT OF PARASITES IN INOCULUM	THE INCUBATION PERIOD IN DAYS				TIME FROZEN IN DAYS		SIZE OF INOCULUM; MILLIONS OF PARASITES PER MM.^{3}	NUMBER OF CASES
	MEAN	MEDIAN	MODE	EXTREMES	MEAN	EXTREMES	EXTREMES	
Young trophozoites	11.9 ± 3.74	11.5	10.7	6-22	44.0	6-131	9-100	20
Mature trophozoites	13.9 ± 2.77	14.0	14.2	10-20	18.0	3-47	9-137	8
Schizonts	12.3 ± 2.37	12.0	11.4	10-16	42.9	9-166	19-46	9

trophozoites and schizonts, the least resistant. If that hypothesis were correct then we would expect more successes and shorter incubation periods when using the younger forms. We have tried to draw blood from donors at times in relation to the developmental cycle to provide young, half-grown, or mature forms of the parasites. However, since merozoites are beginning to be liberated, probably hours before the mass disruption of schizonts, both young and old forms of parasites may be present in considerable numbers near the end of the cycle, and differences in results might be expected to be less from samples taken early and late than from those taken early and mid-way in the cycle.

It is not possible to decide whether age of parasites had any effect on the proportion of successes to failures because the numbers are too few. Successes and failures both followed inoculation of young, middle-aged, or old parasites. When successes alone are considered, there is suggestive evidence that the incubation period was shorter when the inoculum contained a majority of young ring forms than when the majority were mature trophozoites. This is shown in Table III, which indicates that when young ring forms predominated the incubation periods were grouped around eleven days; when mature trophozoites predominated, around fourteen days; and when schizonts predominated with some young trophozoites, the incubation periods were grouped around twelve days. In other respects the groups are fairly comparable, except that the average time frozen was much shorter in the mature trophozoite group.

It is always hazardous to draw conclusions from small differences which may exist between small groups, but apparently there is a significant prolongation of incubation period in the mature over the young trophozoite groups. The difference between the means is 2 ± 0.737, the difference being nearly three times the standard error. The significance of the difference is further borne out by a comparison of results following inoculation of one group of seven patients with blood from donor Mi. with another group of four patients inoculated with blood from Ho. The size of inoculum and duration of preservation were essentially equal and the blood had been citrated in both cases. Blood from Mi. showed parasites approximately forty hours old, schizonts, while that from Ho. taken just at the time of the paroxysm showed a majority of parasites to be very small ring forms, with a number of mature schizonts. In the Mi. group the incubation period averaged 12.7 days, with a range of ten to sixteen days, while in the Ho. group the incubation period averaged 7.7 days with a range of six to ten days.

Anticoagulation.—Citrated blood was used in thirty-two cases with successes resulting in 27 or 84 per cent, and defibrinated, in 14 cases, with 13 or 93 per cent resulting in malaria; this is not a significant difference. In the cases in which malaria developed there was no apparent significant difference in incubation periods in the two groups, which were comparable in other ways.

The speed of freezing and thawing was as nearly identical as possible in all cases and the conditions of storage were the same in all.

The Length of Period of Storage.—There is no evidence that the length of time the parasites are kept frozen affects the final results of inoculation. In comparing successful with unsuccessful inoculations it is found that failures

followed freezing periods ranging from 11 to 137 days, and successes followed freezing periods ranging from 3 to as long as 166 days. Other factors in the two groups were essentially comparable. In considering only successful inoculations, Table IV indicates that the incubation period does not change as the duration of freezing increases.

TABLE IV. THE LENGTH OF STORAGE OF PARASITES IN RELATION TO THE INCUBATION PERIOD AND SIZE OF INOCULUM

STORAGE PERIOD IN DAYS	INCUBATION PERIOD IN DAYS		SIZE OF INOCULUM; PARASITE COUNT IN MILLIONS PER MM.[3]	NUMBER OF CASES
	MEAN	EXTREMES	EXTREMES	
3-10	12.2	9-16	9-64	6
11-20	13.2	10-20	9-137	11
22-28	12.0	10-16	13-225	7
37-59	12.1	6-17	13-100	7
70-166	12.1	8-22	17-69	7
3-166	12.4	6-22	9-225	38

Incubation Period.—In thirty-eight patients who developed malaria, the incubation period, when it could be accurately determined, varied between six and twenty-two days, the mean being 12.4 days. This would seem to be somewhat prolonged over that following the direct transfer method. Moore[3] stated that with the latter using an inoculum of 1 to 10 ml. the average incubation period is three to eight days. We have had only one opportunity to draw a comparison between the two methods. Using direct transfer the incubation period in one recipient was forty hours; with the same blood preserved and inoculated into a different patient, 10 days. It seems likely that the destruction of many plasmodia as a result of the freezing and thawing process, rather than a host factor, was responsible for the prolongation of the incubation period.

The success or failure of attempts to produce malaria in equally susceptible subjects with blood preserved by freezing and the incubation period must depend upon the number of viable parasites surviving. Although available data are insufficient, we have the impression that one important factor in determining the number of parasites surviving is the stage of development, and that merozoites or young trophozoites are more resistant than older forms.

Evidence also has been obtained that infectiousness is not affected adversely by repeated freezing. One strain has been passed serially through six patients, the preservation process being repeated each time, without a significant change in incubation period. Repeated freezing and thawing of the same specimen of blood have not been attempted.

Reactions to Inoculum.—The preservation process results in almost complete hemolysis of the red blood cells; smears after thawing show only an occasional intact cell. The staining properties of the malarial parasites also appear to be affected for, after thawing, the latter are poorly stained by either Wright's or Giemsa's method. Despite these rather marked alterations resulting from the preservation process, no untoward effects directly attributable to the inoculum have been observed following intravenous injection of the relatively small amounts (4 to 10 ml.) employed.

Clinical Course of Malaria.—The clinical course of the disease resulting from inoculations with preserved parasites has in no way differed from that seen following the direct inoculation of whole blood. Striking individual differences in the severity of the disease have been observed, some patients exhibiting a low parasitemia and tolerating the infection well. Others have had fulminating infections, high parasite indices with red blood cells containing as many as three to five parasites, terminating in peripheral circulatory collapse which necessitated interruption of treatment. This individual variation to infection is believed to be related to host rather than to parasite factors. There have been no fatalities in this series of cases.

CONCLUSIONS

1. Preservation of viable parasites of vivax malaria for many months by a simple method of quick freezing, storage at low temperature (–70°), and quick thawing has provided a constantly available supply of parasites in the St. Louis area during the past year.

2. Among forty-six subjects inoculated with preserved blood, forty, or 87 per cent, subsequently developed malaria.

3. The incubation period in those cases of malaria in which an accurate determination was possible averaged 12.4 days, ranging from six to twenty-two days. Malaria was first proved in two additional subjects, forty-five and seventy-eight days after inoculation, although both had slight temperature elevations at ten to fourteen days at which time parasites were not found in blood films.

4. The incubation period probably is directly related to the number of parasites surviving preservation, which in turn appears to be inversely related to the age of parasites; younger forms of parasites are apparently more resistant to freezing and thawing.

5. The clinical course of malaria does not appear to differ in any way from that following infection with fresh parasitized blood.

The authors wish to thank Dr. Louis Kohler, Superintendent of the Missouri State Hospital, and especially Dr. Leopold Hofstatter of the same institution, and Dr. Alfred K. Baur of Jefferson Barracks Hospital for their assistance in providing subjects for inoculation.

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LABORATORY METHODS

SPUTUM CELL STUDY FOR PULMONARY CARCINOMA AS A ROUTINE LABORATORY TEST

BURTON A. SHATZ, M.D., MARTIN BERGMANN, M.D., AND S. H. GRAY, M.D.
ST. LOUIS, MO.

ALTHOUGH the examination of sputum for neoplastic cells as an aid in the early diagnosis of carcinoma of the lung has become well established,[1-11] it is still not being carried out in most laboratories as a routine procedure. The reasons for this hesitancy are understandable. Fear of the harm that might be wrought by a false positive diagnosis, the feeling that specialized cytologic knowledge is required, and the long time frequently necessary for the adequate study of a single case have acted as deterrents. Thus, while there are at present a few laboratories in which this technique for early diagnosis of cancer is being applied very successfully, it is not yet available to most physicians.

To stimulate more widespread use of this valuable diagnostic aid, perhaps a more practical approach to this problem is needed. It has been our experience that the great majority of positive sputum diagnoses are based on cells which have easily recognizable neoplastic characteristics. It was suspected that by confining positive diagnoses only to the relatively obvious cases, the sputum examination might prove to be more adaptable to routine use in a clinical laboratory without fear of false positive diagnoses, and at the same time retain a useful degree of accuracy.

To test this suspicion, a group of forty consecutive cases of proved carcinoma of the lung, in which sputum examinations were done, were analyzed. It was found that in twenty-nine cases (72 per cent) the sputum contained obviously malignant cells. While this percentage of positive results is lower than that reported by some authors,[4, 6] it is still high enough to be of distinct clinical value. Thus, in seven of the twenty-nine cases in which we demonstrated neoplastic cells in the sputum, bronchoscopy had been entirely negative, and histologic proof of the suspected presence of a lung tumor could not be secured by any other means short of exploratory thoracotomy. It would appear that by restricting positive diagnoses only to those patients whose sputa contain easily identifiable malignant cells, and by reporting all others as negative, the sputum examination should become practical for more general adoption. This paper outlines the technique of preparing sputum slides, describes and illustrates the cellular elements encountered, and sets forth the characteristics of the obviously malignant cells.

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TECHNIQUE

The technique has been described in other publications,[2, 10] but certain points bear emphasis. The proper collection of the specimen is one of the most important steps in the sputum examination. Due care exerted in this part of the procedure will be rewarded by noticeably better results and will save the examiner a great deal of time spent in unfruitful search of specimens containing almost no bronchial secretions. The patient must be instructed to cough up material from the tracheobronchial tree and must not be allowed to present the examiner with saliva or nasopharyngeal secretions as a specimen. Material from the patient's bedside sputum cup is usually not satisfactory, due to the dilution of whatever bronchial secretions there are with a relatively large amount of saliva. We have found it advisable to have a well-instructed technician or nurse obtain the specimen directly from the patient.

Storage.—It is best to smear and fix the specimen as soon after collection as possible. However, when necessary, a specimen may be allowed to remain in the refrigerator for four to six hours without appreciable deterioration.

Preparation of Slides.—The specimen is poured into a Petri dish placed on a black background. An effort is made to find small fragments of tissue and blood-tinged material. Samples of the various components of the sputum are selected and placed on a glass slide. A fairly thin and uniform film is obtained by smearing the material with a second glass slide. Ordinarily five slides are prepared from each specimen.

Fixation.—Fixation of the film is accomplished by immersion for at least thirty minutes in a mixture of equal parts of ether and 95 per cent alcohol. It is important for the preservation of cellular details that no drying of the film be allowed to occur before it is fixed. The slides should be placed in the fixative while still wet.

Staining.—The stain employed is hematoxylin and eosin. This stain presents two important advantages over other stains that have been advocated for use in this work. First, it allows the staining of sputum slides to be carried out simultaneously and by the same technique as other routine slides. Second, it relieves the pathologist of the necessity of familiarizing himself with a new stain. From the ether-alcohol mixture the sputum slides are transferred to 95 per cent alcohol for one minute and from then on are handled as are ordinary tissue sections. In our laboratory this consists of the following:

1. Water for one minute
2. Harris hematoxylin for one minute
3. Water for one minute
4. Dip in acid alcohol
5. Water for one-half minute
6. Ammonia water till blue
7. Water for one-half minute
8. One-half per cent aqueous eosin for two minutes
9. Dip in water
10. Dip in increasing concentrations of alcohol to absolute alcohol
11. Beechwood creosote for five minutes
12. Xylol five minutes

The slides are then coverslipped with the use of Clarite. The preparation of the slides, fixation, and staining can easily be handled by a technician. The examination of the slides consists of scanning with the low-power objective, using a mechanical stage so that all parts of the preparation are seen. Suspicious elements are studied with the high-dry objective.

NORMAL CELLS IN SPUTUM

All sputum specimens, regardless of the underlying pulmonary disease, contain normal cells which can be classified according to their place of origin.

Cells From the Blood.—The erythrocytes and leucocytes encountered in the sputum are as easily identifiable as in the usual tissue sections. Occasionally, when the sputum is purulent, the number of leucocytes is so great as to obscure the other cellular elements. In these instances it has been our policy to recommend a course of penicillin therapy to reduce the number of inflammatory cells and thereby facilitate proper sputum examination.

Cells From the Mouth and Pharynx.—(Plate I, *1*.) These cells arise from squamous epithelium and are large and polygonal in shape. They contain an abundant amount of pale pink cytoplasm, with small centrally located nuclei. The nucleus is usually light blue, round to oval in shape, and surrounded by a distinct nuclear membrane. It has a finely granular chromatin network and may have a small distinct nucleolus. The cytoplasm may contain numerous invading bacteria. These squamous epithelial cells may occur singly or in sheets. The number of these cells present is a good index of the amount of saliva contained in the sputum. Their identification offers no difficulty.

Cells From the Epithelium of the Tracheobronchial Tree.—(Plate I, *2* and *3*.) The tall ciliated columnar cells which line the trachea and bronchi are frequently present in the sputum and may occur singly or in clumps. The basal end may be drawn out into a long filamentous process. The nucleus is basally located, homogeneous in appearance, and stains deep blue. As many as three nuclei have been observed in a single cell. When these columnar cells occur singly and are not influenced by the presence of surrounding cells, they may assume a spherical shape. The ciliated border can be made more distinct by decreasing the aperture of the substage condenser. Goblet cells may be seen in the sputum. They can be recognized by the presence of a large mucigen droplet in the distal part of the cell, as seen in Plate I, *3*.

Macrophages.—(Plate I, *4*, *5*, *6*.) These are large round cells which may occur singly or as clusters of individual cells and are found in almost all sputa. They contain moderate-sized nuclei and abundant cytoplasm. The nuclei may be round, oval, or bilobed and usually have small nucleoli. However, at times the nucleolus is quite prominent. The chromatin is rather evenly distributed throughout the nucleus. Occasionally multinucleated giant macrophages are seen. The cytoplasm may be clear or may contain a variety of ingested particles and vacuoles. The blood pigment-containing cells (heart failure cells) have been found to be associated not only with chronic passive congestion of the lungs, but may also be found in a variety of conditions, especially those in which hemoptysis has occurred.

NEOPLASTIC CELLS

There are certain characteristics which most cancer cells have in common, regardless of the type of tumor from which they arise. These are as follows: (1) Neoplastic cells tend to occur in groups or tumor fragments. (2) Marked hyperchromasia of the entire cell is an outstanding characteristic and serves to make the malignant cells conspicuous even under low power magnification. (3) There is complete loss of polarity in the arrangement of neoplastic cells in a tumor fragment. (4) There usually is marked variation in the size and shape of malignant cells and their nuclei in any one tumor. (5) The nuclei of tumor

cells are generally large relative to the size of the cell. Multiple nuclei are not uncommon. (6) The nucleoli are large, distinct, often multiple, and may take a pink stain in contrast to the dark blue color of the nuclei. (7) Malignant cells may be phagocytic and it is not unusual to find one malignant cell ingested by another (Plate IV, *18*). (8) Neoplastic cells may contain large, deep blue cytoplasmic bodies, which are known as bird's-eye inclusions of Leyden (Plate IV, *20*). The nature of these bodies has not been established, but it is thought that they probably arise from the multiplication of centrosomes, or that they are retained secretory products of the cell.[3] The neoplastic cells in the sputum fall into two major categories: undifferentiated and classifiable.

1. *Undifferentiated Cells.*—(Plate II, *9.*) These are tumor cells which possess only the described general characteristics. They arise from a totally undifferentiated tumor or from undifferentiated portions known to occur commonly in squamous carcinoma, adenocarcinoma and small-cell carcinoma. Therefore, undifferentiated neoplastic cells in the sputum may or may not reflect the predominating histologic character of a tumor. Conversely, a histologically differentiated tumor can give rise to both undifferentiated and classifiable elements in the sputum.

2. *Classifiable Cells.*—These cells, in addition to the general characteristics of neoplastic cells, have certain distinguishing features which allow one to classify them as to the type of tumor from which they arise. (a) Squamous carcinoma cells (Plate II, *10*, and Plate III, *11*, *12*). The squamous carcinoma cells tend to be elongated in shape. The nuclei are usually oval to spindle-shaped, but may be round, and have a dense, coarsely granular chromatin structure. Nucleoli may be single or multiple and are usually very prominent, large and pink-staining. The two characteristics that are diagnostic of this type of tumor are keratinization of the cytoplasm, which imparts to it a deep-pink to red color, and the arrangement of the tumor cells in whorls. True epithelial pearls are not uncommon. (b) Adenocarcinoma cells (Plate III, *13*, *14*). Cells of this type are usually rather large and round or oval in shape. The nuclei are round, frequently multiple, and commonly contain one or more small discrete nucleoli. Occasionally single large nucleoli are seen. The cytoplasm is usually abundant and several small, or one or two large, clear droplets of secretion are present. Secretory vacuoles are the only valid criterion for the diagnosis of adenocarcinoma unless one finds malignant cells forming true acini. This arrangement, however, is quite rare. (c) Small-cell carcinoma (Plate III, *15*). Small-cell carcinoma cells are the smallest of the malignant cells in the sputum. They are round or elongated in shape and of fairly uniform size. The nucleus fills the cell almost completely, so that only a narrow rim of cytoplasm is detectable. The nucleus is finely granular; moderate-sized single nucleoli are the rule. To the untrained eye these cells resemble large lymphocytes, except that they occur as fragments of tissue.

DISCUSSION

In applying the described criteria for malignant cells to the examination of sputum, one must be aware of certain pitfalls which may cause erroneous diagnoses. Overstaining with hematoxylin may so obscure the structure of the

nuclei as to render adequate study difficult and to make it impossible to appreciate hyperchromasia, because all the nuclei, benign or malignant, will appear dark blue. One of the important criteria of malignancy is thus lost.

Another possible source of error may be the presence of degenerating normal cells. The changes brought about by this process include hyperchromasia and swelling of the nucleus, vacuolization of the cytoplasm, and changes in size and shape of the cell. Degenerating normal cells may thus assume what appear to be characteristics of malignancy. But careful observation will disclose loss of distinct cellular and nuclear outlines and loss of nuclear detail. Frequently, in the close vicinity one finds similar but better preserved benign cells, and others which are obviously and more completely degenerated. Occasionally macrophages which have no visible ingested material in their cytoplasm and whose nucleoli are rather prominent are encountered. Such cells may simulate undifferentiated neoplastic cells, or, if they contain vacuoles, cells arising from adenocarcinoma. However, phagocytes occur only as individual cells and are not hyperchromatic, and similar cells with ingested material can usually be found in the immediate vicinity.

In chronic infectious diseases the normal bronchial epithelium may undergo squamous metaplasia (Plate I, 7). Fragments of the metaplastic epithelium are frequently exfoliated into the sputum and may resemble bits of well-differentiated squamous carcinoma. However, these cells are arranged in an orderly fashion, have a definite polarity, and usually do not contain visible nucleoli. As a result of degeneration, they may show both hyperchromasia and variation in size and shape of the nuclei. However, the cause of these characteristics becomes evident when one notes the loss of nuclear structure, distinct nuclear outline, and vacuolization of the cytoplasm.

Occasionally one is tempted to make a positive diagnosis on the basis of one or two single cells which have all the characteristics of malignancy. It is wise in such instances to withhold the final diagnosis until further specimens can be examined. In almost every case one will eventually find either numerous other similar cells or small fragments of tumor tissue. It has been shown that the examination of three separate sputum specimens will yield about 96 per cent of the positive diagnoses.[9] However, if the clinical history and roentgenographic studies strongly indicate malignancy, and especially when bronchoscopic biopsy is impossible, as in upper lobe lesions,[1] it is often desirable and worth while to repeat the examination several more times.

The diagnosis given to the clinician should be concise and explicit. We feel that the grouping of cells as to the probability of malignancy is confusing and even dangerous. Unless the pathologist is absolutely certain that carcinoma cells are present in the sputum, he should render a negative report. Care should be taken to advise the clinician as to the significance of positive and negative reports. The presence of cancer cells in the sputum is not a priori evidence of primary bronchogenic carcinoma. The cells may arise from carcinomas of the mouth, pharynx, nasopharynx, larynx, esophagus, trachea, or from pulmonary metastases, and in such instances cannot be differentiated from cells of primary bronchogenic neoplasms. Above all, one must be aware of the fact that failure

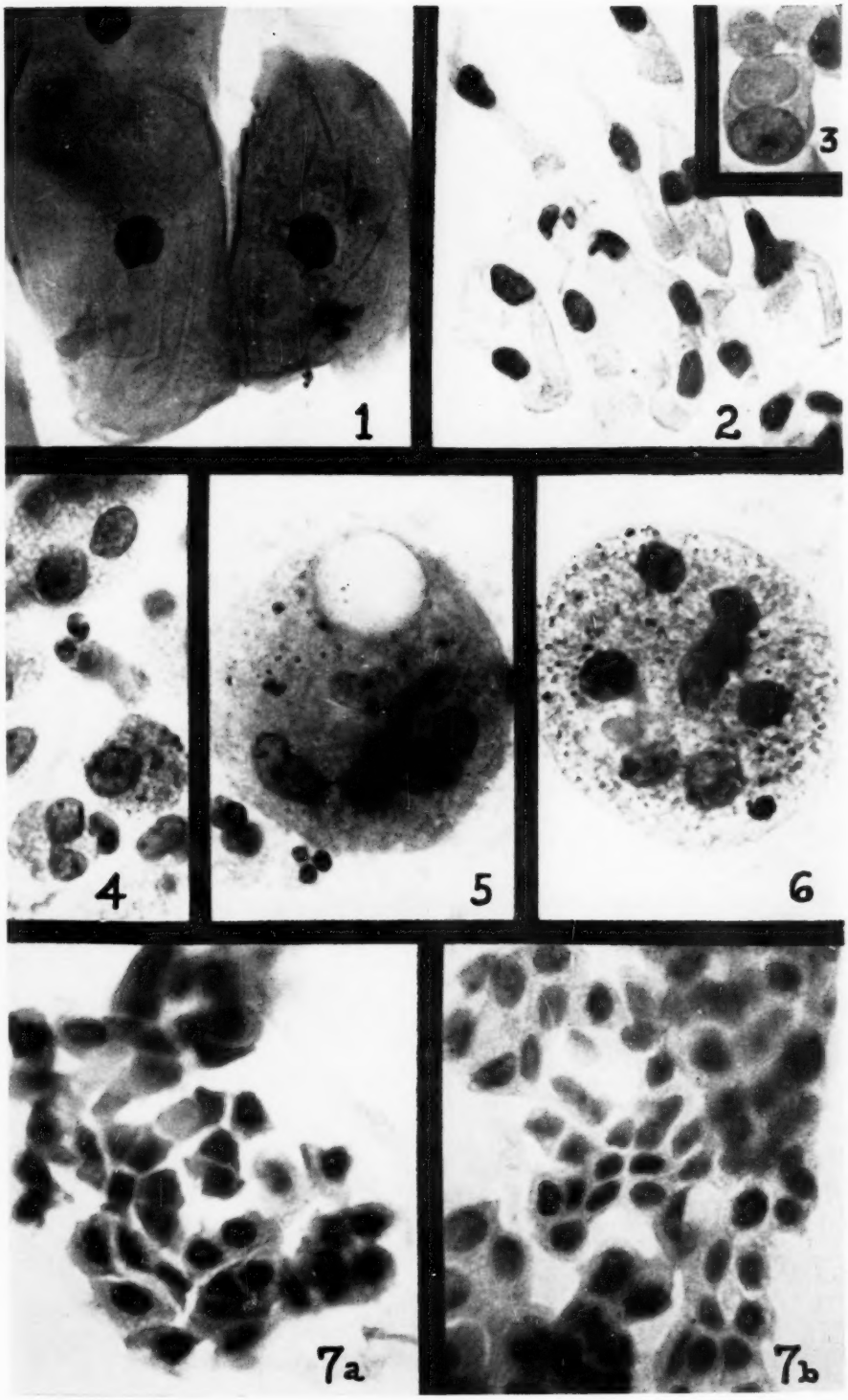

PLATE I*

1, Squamous epithelial cells.

2, Bronchial epithelial cells.

3, Goblet cell with large mucigen droplet in the cytoplasm.

4, Macrophages, some with ingested particles.

5, Giant multinucleated macrophage with cytoplasmic vacuole. Note neutrophile for comparative size.

6, Giant multinucleated macrophage with numerous ingested particles.

7 a and *b*, Squamous metaplasia of bronchial epithelium with degenerative changes. From cases of bronchiectasis.

*All photographs were taken under oil immersion lens (×1320) except where noted.

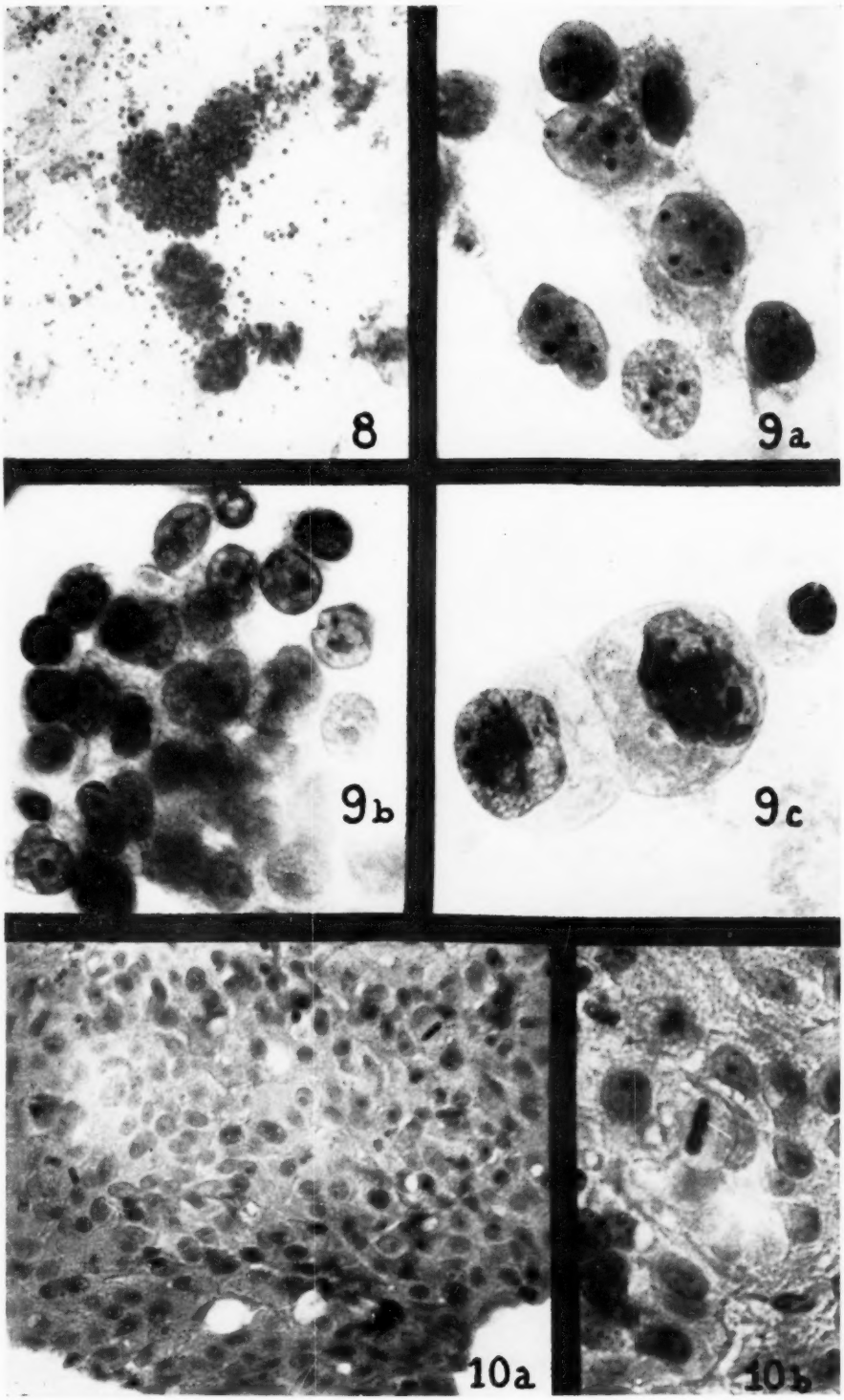

PLATE II

8, Conspicuous hyperchromatic tumor fragments as seen under low-power magnification (×140).

9, Undifferentiated cells from: (*a*) Undifferentiated bronchogenic carcinoma.
(*b*) Squamous carcinoma.
(*c*) Adenocarcinoma.

10, Squamous carcinoma. (*a*) Large fragment of tumor (high-dry magnification: (×610).
(*b*) Mitotic figure.

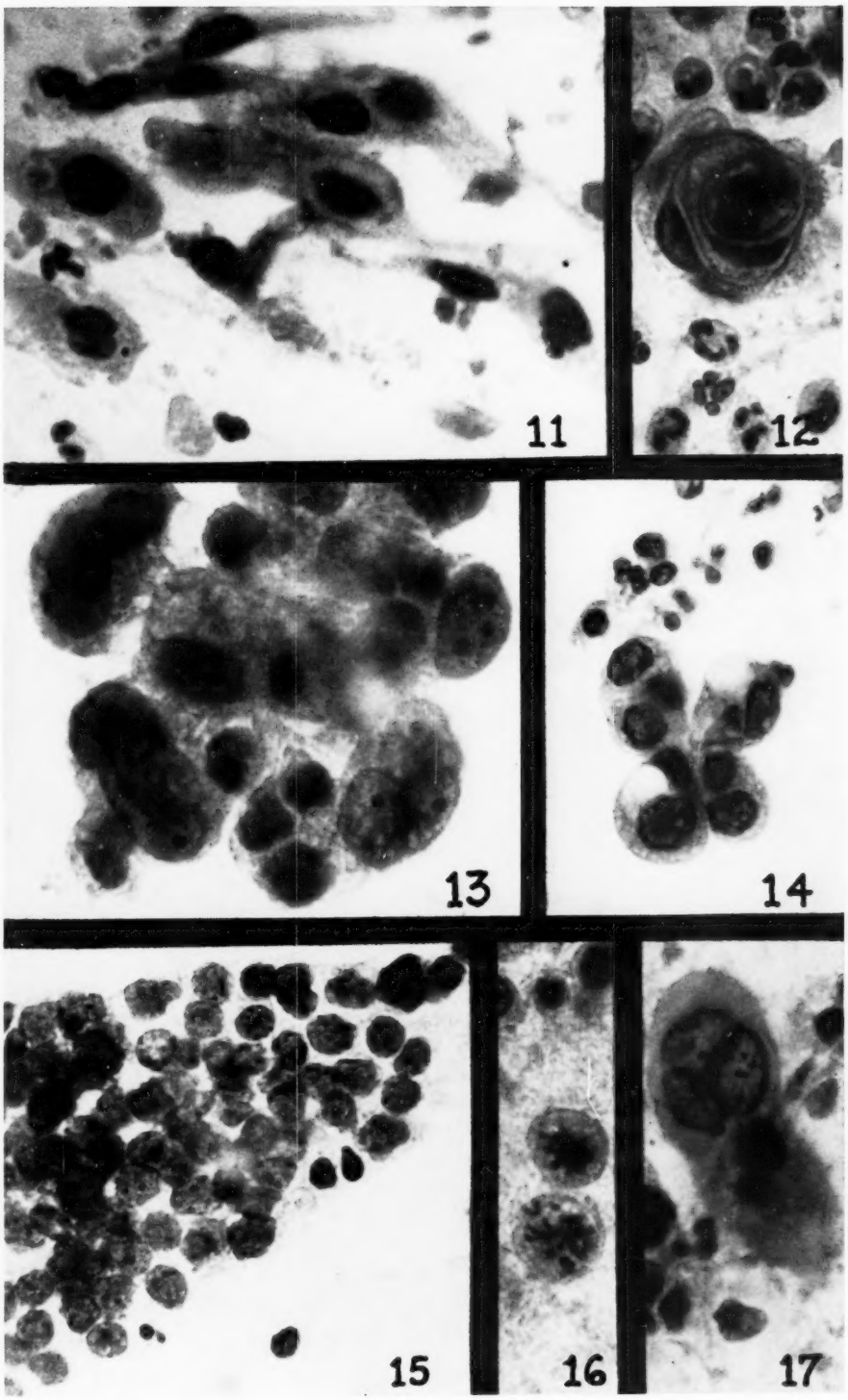

PLATE III

11, Squamous carcinoma. Note elongated large tumor cells.
12, Squamous carcinoma. Epithelial pearl.
13, Adenocarcinoma. Note multinucleated cells and vacuolated cytoplasm.
14, Adenocarcinoma. Cells with secretory vacuoles.
15, Small-cell carcinoma. Note resemblance to lymphocytes.
16, Undifferentiated carcinoma. Two mitotic figures.
17, Squamous carcinoma. Atypical nuclear division.

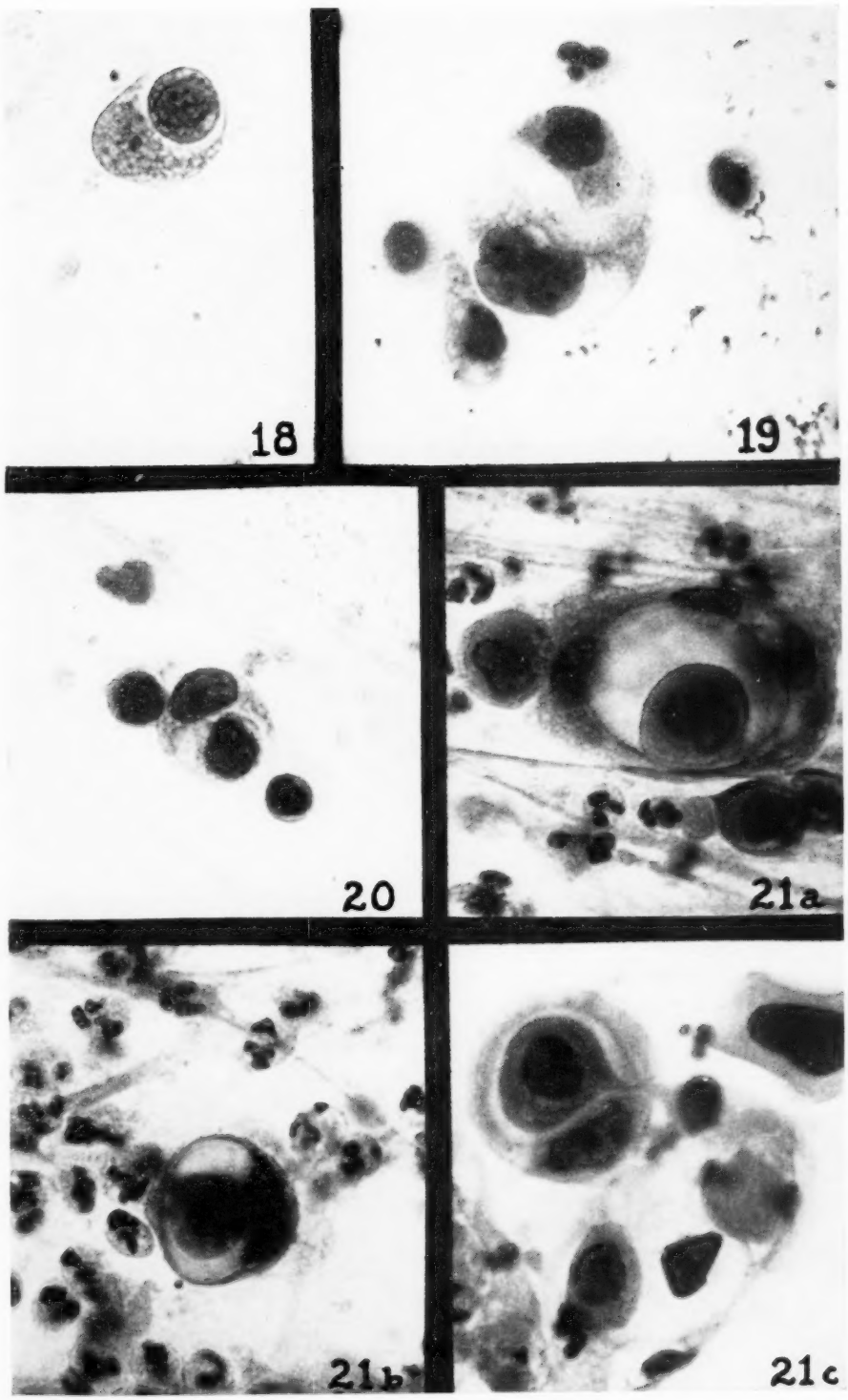

PLATE IV

18, Adenocarcinoma. One tumor cell phagocytized by another.

19, Adencarcinoma. Tumor cells phagocytized by large macrophage. Note pale nucleus of macrophage just below upper group of ingested malignant cells.

20, Adenocarcinoma. Bird's-eye inclusion.

21 a, *b*, and *c*, Squamous carcinoma. Unusual configurations of tumor cells.

to find malignant cells in the sputum does not rule out carcinoma and should be disregarded if the weight of other clinical or radiologic evidence favors this diagnosis.

CONCLUSION

Easily recognizable malignant cells occur in the sputa of more than 70 per cent of cases of pulmonary cancer. When one restricts positive diagnoses to these comparatively obvious cells the danger of false positive diagnoses is reduced to a minimum while at the same time the test retains a good degree of accuracy. With the realization of these facts, sputum examination becomes more suitable for widespread adoption as a routine laboratory procedure.

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A MICROADAPTER FOR THE EVELYN MACROCOLORIMETER

ESTHER F. FREIER, B.S., AND EVREL A. LARSON, M.D., PH.D.
MINNEAPOLIS, MINN.

THE Evelyn macrocolorimeter[1] requires the use of a minimum volume of 6 c.c. of solution in order to obtain a satisfactory colorimetric reading. It would be advantageous in some clinical procedures, such as the Evans blue dye blood volume method, to obtain a reading on a much smaller volume. The Evelyn microcolorimeter,[2] which allows colorimetric readings with volumes of solution ranging from 0.1 to 2.0 c.c. is technically difficult to operate; therefore, its application to routine laboratory procedures is limited.

In order to obviate the inherent difficulties of the Evelyn microcolorimeter, the microadapter described here was designed to permit readings on volumes as low as 1 c.c. with the Evelyn macrocolorimeter. Flat-bottomed microcolorimeter tubes,* 10.8 mm. in inside diameter, can be used with the adapter, in the Evelyn macroinstrument.

Construction.—The adapter, shown in Fig. 1, is constructed of bakelite The outside diameter is 22 mm. which permits easy removal from and insertion into the bakelite tube sleeve of the Evelyn. The projection on the bottom of the adapter fits into a slot cut in the inside bottom of the tube sleeve and locks the adapter in place with its aperture opposing the 6 c.c. aperture of the tube sleeve.

The aperture of the adapter is cut slightly larger than that of the tube sleeve, so that the sleeve aperture determines the light path. The inside diameter of the adapter, 12.3 mm., was chosen to accommodate the microtubes. The cut-outs along the side of the adapter permit the operator to guide the tube, which is 4½ inches in length, as it is put into or taken from the adapter. The adapter has an over-all length of 7 and 7/16 inches including the 3/16 inch projection.

Performance.—The microtubes are calibrated for equality of transmission in the same manner as Evelyn tubes. To obtain a center setting the adapter need be only partially removed until clear of the light beam through the sleeve aperture; this maneuver permits rapid checks of both center settings and readings.

Readings were made with the Evelyn macrocolorimeter with and without the adapter, using the same Evans blue solutions in serial concentrations from 0.0015 mg. per cubic centimeter to 0.0105 mg. per cubic centimeter. One series of results is presented in Table I. The "L values" (photometric density) obtained with the adapter were multiplied by 19.5/10.8 (the ratio of the diameters of the Evelyn and microtubes) in order to compare the two sets of readings. It may be noted that density values obtained with the adapter, when corrected in this manner for the difference in diameter, agree quite closely with those obtained without the adapter.

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*Available from Hellige, Inc., Long Island, N. Y., under the name Hellige-Diller Micro Tubes.

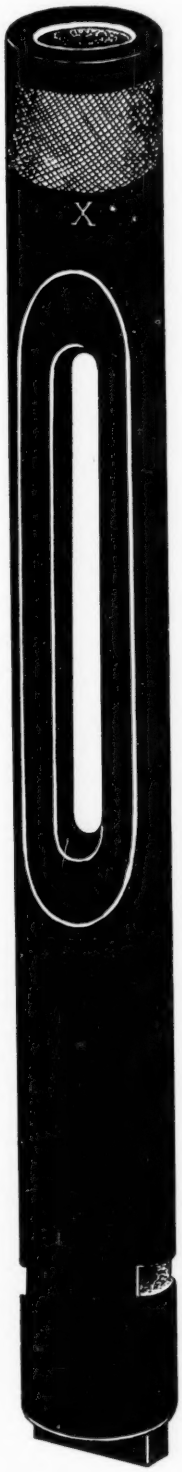

Fig. 1.—An oblique view of the adapter.

TABLE I. COMPARISON OF EVELYN MACROCOLORIMETER READINGS WITH AND WITHOUT ADAPTER

CONCENTRATION OF DYE SOLUTION (MG./C.C.)	READING WITHOUT ADAPTER	L VALUE WITHOUT ADAPTER	READING WITH ADAPTER	L VALUE WITH ADAPTER	L VALUE WITH ADAPTER CORRECTED FOR DIFFERENCE IN DIAMETER OF TUBES
.0015	85[3]	.0667	91[3]	.0374	.0675
.0030	74[3]	.1278	85	.0706	.1275
.0045	66[2]	.1772	79[3]	.0982	.1772
.0060	57[2]	.2384	73	.1367	.2434
.0075	51	.2924	67[3]	.1691	.305
.0090	45	.347	64	.1922	.347
.0105	40	.398	59	.2291	.412

In addition, for any given concentration of Evans blue solution, the adapter gave the same reading with a volume of 1, 2, or 5 cubic centimeters. It appears that use of the adapter successfully increases the volume range of the Evelyn macrocolorimeter from 6 c.c. down to 1 cubic centimeter.

SUMMARY

A microadapter for the Evelyn macrocolorimeter has been described which permits readings on volumes of solution as low as 1 cubic centimeter. Evidence has been presented to substantiate the validity of results obtained with the adapter.

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VENOUS CATHETERIZATION WITH POLYETHYLENE TUBING

SAMUEL L. CRESSON, M.D.,* AND WILLIAM W. L. GLENN, M.D.†
PHILADELPHIA, PA.

THE use of polyethylene tubing in the catheterization of veins has been reported by Zimmermann[1] and Meyers.[2] It is the purpose of this communication to describe and evaluate a method of taking samples of portal vein blood in the ambulatory dog, and to record some of the observations made on the intimal reaction to the plastic polyethylene.‡

TECHNIQUE

Under general anesthesia with 5 per cent Nembutal, a transverse right subcostal muscle splitting gridiron incision is made The peritoneum is dissected free by blunt dissection and reflected medially. The portal vein is approached retroperitoneally and is then followed cephalad toward the hepatic border. The peritoneum is frequently opened just as the portal vein is approached. A site is selected just cephalad to the mesenteric radicals in the main trunk of the vein. The loose areolar tissue is dissected away, exposing the vein wall. A purse-string suture of fine black silk is placed, but not tied, in the vein wall. Then a No. 16 gauge needle is thrust through the center of the purse-string suture, with the end of a small caliber polyethylene tube about 12 inches long inside the needle bore, but not projecting beyond the needle bevel (Fig. 1). Tubing with an internal diameter of 0.023 inch has been found most satisfactory. After piercing the vein wall, the cannula is pushed through the needle bore into the lumen of the vein for about 3 centimeters. The needle is then pulled out of the vein and the polyethylene tubing is held stationary by a pair of thumb forceps. As the needle is pulled clear of the plastic tubing, the purse-string suture is tied. The plastic tubing is then aspirated. If aspiration is adequate, several loops of the suture are placed around the tubing, slipped down next to the vein wall and tied again. Sterile saline is injected into the tubing and the end of the tubing sealed by heating the tip of the tubing and then pinching it with forceps while still hot. The same procedure may be used for inferior vena cava catheterization.

We have used as a criterion of an adequate catheterization the aspiration of 5 c.c. of blood in thirty seconds.

The tubing is brought out retroperitoneally through a separate stab wound and the incision closed. The tubing external to the skin is coiled and is held in place with a piece of gauze saturated with collodion. A firm body-encircling adhesive dressing is then applied.

RESULTS

We have performed twenty-four portal vein catheterizations in twenty dogs. The first attempt at aspiration of the vein catheter usually was made seven days after insertion. In four, no blood could be aspirated on the first or subsequent trials. In eleven, either the animal pulled the tubing out, or the tubing fell out, or the dog died before aspiration was attempted. A number of the failures could

From the Department of Surgical Research, Jefferson Medical College.
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*Resident in Surgery, Pennsylvania Hospital.
†Associate in Surgery, Jefferson Medical College.

‡The polyethylene used in these experiments was purchased from the Surprenant Electrical Insulating Company, Boston, Mass. It was furnished us as *pure* polyethylene without the addition of a plasticizer.

be attributed to preventable errors in technique. The remaining nine catheterizations functioned well for an average of twenty-one days after catheterization. The maximum time adequate blood samples were obtained from the portal vein was thirty-four days. In several animals the portal vein was successfully catheterized two or more times at intervals of several weeks. In only one animal did

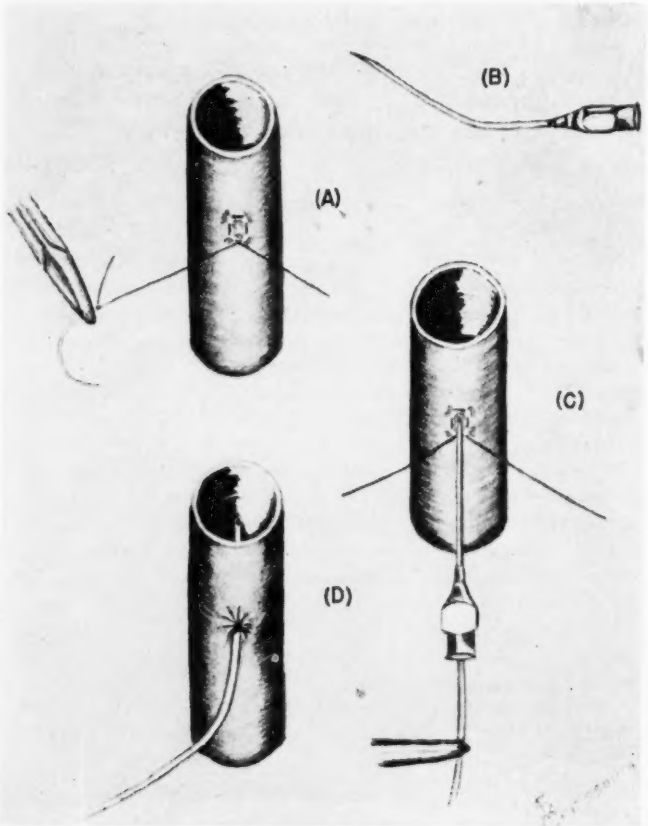

Fig. 1.—Technique of insertion of fine polyethylene tubing into portal vein lumen.

complete thrombosis of the portal vein result, and this followed three separate catheterizations of the portal vein at two- to three-week intervals, the last tube having been left in the vein for two months.

Although adequate specimens of blood were aspirated for prolonged periods in 37.5 per cent of the experiments, some degree of reaction to the polyethylene tubing was noted in every case where the portal vein was examined.

The first reaction to the polyethylene tubing was the formation of a thrombus surrounding the intraluminal portion of the tubing (Fig. 2). Later, fibrous tissue was laid down over the tube with puckering of the adjacent intimal lining. (Fig. 3.) These events took place in from two to four weeks after the tubing was inserted into the vein lumen. In one instance the tubing was completely excluded from the vein lumen by fibrous tissue at three weeks. The tip of the tubing remained free in most cases, however, although an area of granulation

was sometimes seen on the intima adjacent to the free tip of the tubing. Attempts to obtain blood samples over periods of several days from veins of small size catheterized with polyethylene tubing were unsuccessful. It is evident from Figs. 2 and 3 that the lumen of a small vein may become occluded promptly.

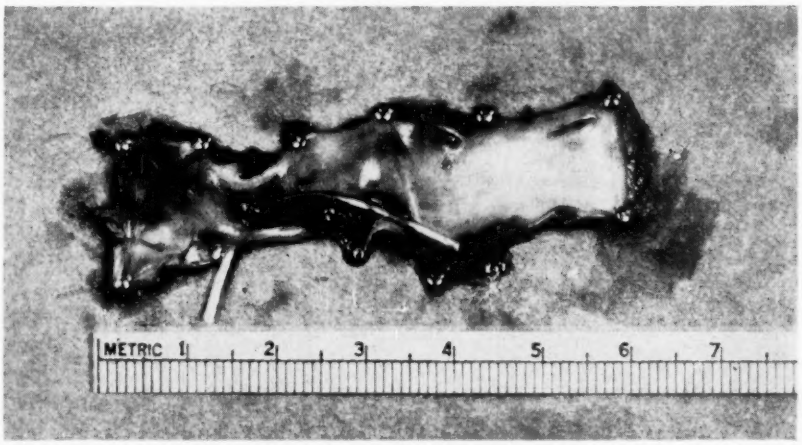

Fig. 2.—Polyethylene tubing inserted into portal vein four days prior to sacrifice of the dog. Note the thrombus formation surrounding the intraluminal portion of the tubing.

Fig. 3.—Polyethylene tubing inserted into portal vein twenty-two days prior to sacrifice of the dog. Note coiling of excess tubing; arrow points to open end. Scarring and puckering of intimal lining around polyethylene tubing. Samples of portal blood were aspirated through tubing for fifteen days.

DISCUSSION

It is apparent from these experiments that catheterization of large veins may be carried out with polyethylene tubing of small caliber and that satisfactory blood samples may be obtained over a period of approximately three weeks.

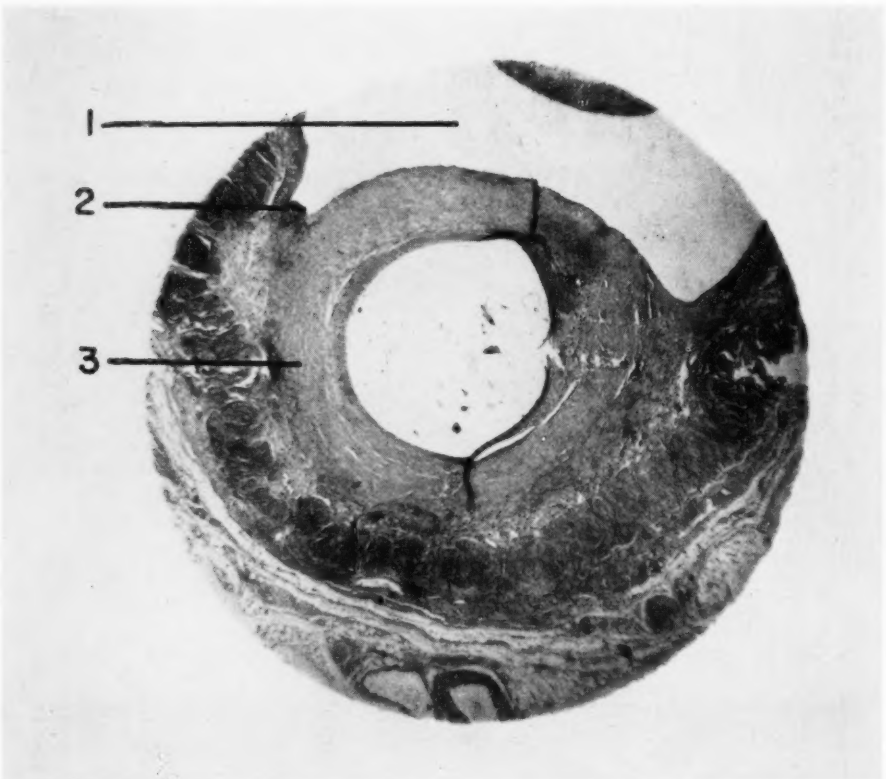

Fig. 4A.—Microphotograph of portal vein ×16. Polyethylene tube inserted into vein twenty days prior to sacrifice of dog. *1*, Lumen of vein. *2*, Junction of intimal surface of vein wall and tissue overlying polyethylene tube. See Fig. 4*B*. *3*, Fibrous tissue surrounding space where polyethylene tube lay. Tube dislodged or dissolved during preparation of histologic section.

Repeated catheterizations are possible although there is evidence that increasing damage to the vein wall results. The use of polyethylene tubing is an improvement over the London cannula[3] for larger vessels but is not satisfactory for vessels of small size.

There has been some controversy over the tissue reaction to polyethylene. Ingraham and co-workers[4] emphasized the slight tissue reaction to this plastic in the pure form. They believed that the marked fibrous tissue response noted by Poppe and de Oliveira[5] to sheets of polyethylene was due to the presence of a plasticizer. For this reason Ingraham and associates[4] insisted on *pure* polyethylene to which they found only slight reaction when it was buried in cerebral tissue of various experimental animals. Zimmermann[1] did not indicate whether he used polyethylene in the *pure* form, but it is of interest that in two of the four experiments where the veins were examined, thrombosis was found. He was not certain whether this reaction was due to the tubing per se or to the infused fluid. There was no infusion of fluids in any of the experiments recorded here except the small amount used to flush the tubing out at the beginning and end of each aspiration. The reaction to pure polyethylene when placed in the lumen of a blood vessel may, however, differ from the reaction in soft tissues

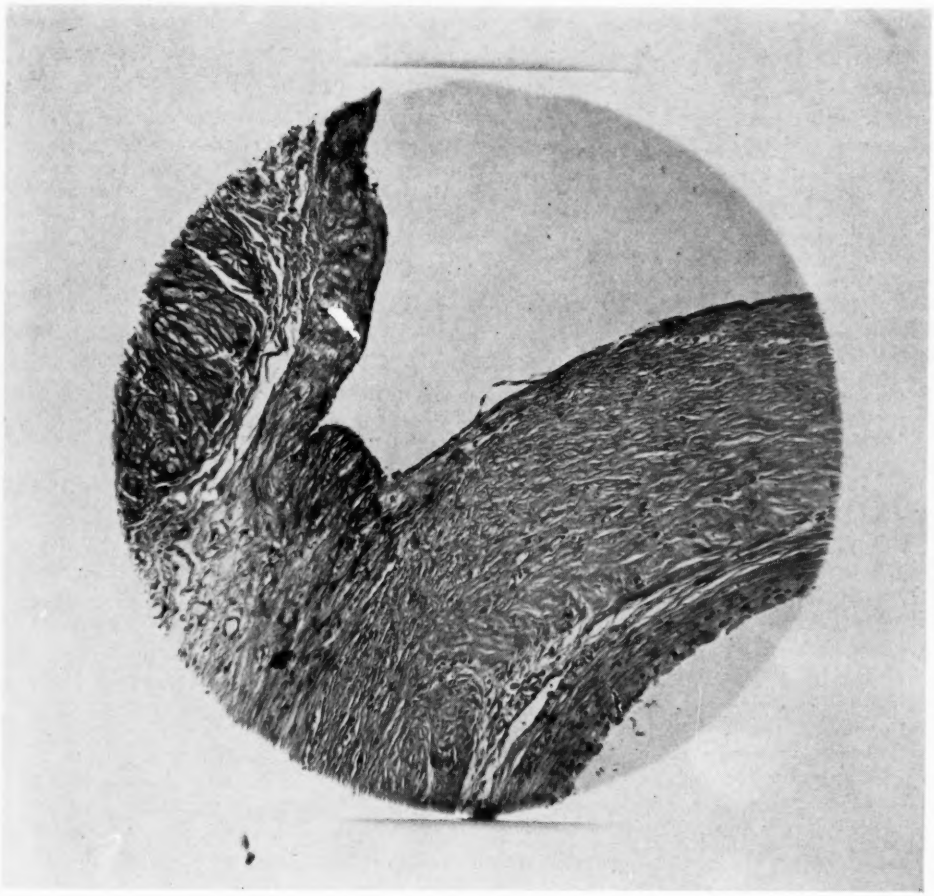

Fig. 4*B*.—Microphotograph of junction of vein wall and fibrous tissue surrounding polyethylene tube. Tube is no longer present. (×100.)

due to the formation and subsequent organization of a blood clot surrounding the tubing. It was our impression that the fibrous tissue reaction to the tubing was progressive and that the longer the plastic remained in the lumen of the vessel the more pronounced the reaction became (Figs. 4*A* and 4*B*). This suggests the possibility of using this material intraluminally for the gradual occlusion of arteries.

SUMMARY

The catheterization of the portal vein for the purpose of obtaining blood samples from the ambulatory dog over prolonged periods can be satisfactorily performed with small caliber polyethylene tubing. The retroperitoneal approach to the portal vein lessens the period of postoperative discomfort and renders the animal available for study at an earlier time. The inferior vena cava is exposed simultaneously by this approach and may also be catheterized.

The presence of pure polyethylene in the lumen of a vein results in a fibrous tissue response that tends to exclude the tubing from the lumen of the vessel

with narrowing of the lumen of the vein. It is not clear how much of the fibrous tissue reaction is due to the organization of the thrombus that surrounds the tubing. The fact that the fibrous tissue reaction appears to be progressive, however, would seem to indicate that polyethylene per se possesses some irritative action in blood vessels.

The authors wish to acknowledge the helpful advice of Dr. C. Martin Rhode and Dr. William M. Parkins of the Harrison Department of Surgical Research, University of Pennsylvania.

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SCREW SYRINGE*

ARNOLD LAZAROW, M.D., PH.D.
CLEVELAND, OHIO

A METHOD for carrying out microcolorimetric capillary tube analysis has been described using pipettes similar in construction to the white blood cell counting pipettes.[1, 2] The screw syringe was devised for more precise control of the flow of fluid within the capillary tubes. This screw syringe also can be used to improve the accuracy of diluting blood for cell counts, for controlling the flow of liquids in micropipettes (.001 to .1 c.c.), and for controlling the flow of radioactive and other toxic agents within ordinary pipettes, thus avoiding the danger of sucking noxious materials into the mouth.

CONSTRUCTION

The syringe consists of the following parts: (Figs. 1 and 2) A rubber collar *(A)* contained within the collet *(B)* fixes the pipette firmly in place when the collet is tightened. The body of the control device *(D)* contains the plunger *(F)* as well as the screw control (E) for this plunger. Two doughnut-shaped "O" rings *(I* and *J)* seal the plunger within the body *(D)* and within the screw control *(E)*. Because "O" ring *(J)* is compressed more than is "O" ring *(I)*,† the plunger *(F)* will turn with the control screw *(E)* upon rotating the latter, and this provides the delicate control of the liquid within the pipette. However, the plunger can be withdrawn directly from the body without the use of the control screw *(E)*, and this provides a coarse adjustment for the fluid volume. An air vent avoids the displacement of the liquid within the pipette when the latter is removed from the collet. The "O" ring *H* is placed within the body of the syringe and seals the vent hole *(G)*, when the collar *(C)* is loose. When the collar *(C)* is tightened, the "O" ring is distorted, the hole in the body *(D)* is uncovered, and the vent is open. The capacity of the screw syringe is 3 cubic centimeters.

USE OF CONTROL DEVICE FOR BLOOD COUNTING AND BLOOD CHEMISTRY MICROPIPETTES

The collet, containing an appropriate rubber adapter, is attached to the syringe body; the pipette is inserted and fixed in place by tightening the collet; the air vent is closed by loosening the collar *(C)*; and the apparatus is ready for use. By means of control screw (*E*, Fig. 3), a drop of blood is sucked into the stem of the pipette slightly above the mark; the excess blood is wiped off the tip of the pipette, and the blood is adjusted precisely to the mark by simultaneously rotating the control screw and wiping the tip of the pipette on the operator's finger. The tip of the pipette is then placed in diluting fluid (Fig. 4) which is sucked into the bulb of the pipette by withdrawing the plunger *(F)*. As soon as the meniscus approaches the upper capillary tube of the pipette, the screw control *(E)* is used to accomplish the final exact adjustment. The pipette is re-

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*The screw syringe is available from the Micrometric Instrument Company, Cleveland, Ohio.

†The use of the differential "O" ring pressure was suggested by A. Howard of the Micrometric Instrument Company.

moved from the diluting fluid and the tip carefully wiped with the finger or Kleenex. (If Kleenex is used, one must avoid placing it against the tip of the capillary in order to avoid withdrawal of fluid by capillary action.)

The index finger of the left hand is placed over the tip of the pipette, and while holding the bulb of the pipette between the thumb and middle finger, the body of the screw syringe is rotated with the right hand, and the pipette is

Fig. 1.

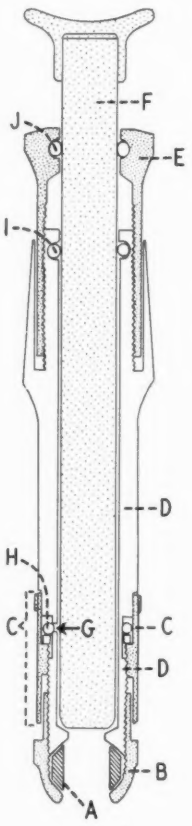

Fig. 2.

Fig. 1.—Screw syringe with micropipette inserted.
Fig. 2.—Construction of screw syringe.

removed. The finger seal prevents the displacement of the fluid within the capillary during removal. An alternate method for removing the pipette from the screw syringe utilizes the vent. The pipette and its holder are placed in a near horizontal position; the air vent is opened; the collet is loosened; and the pipette is removed. (If placed in an absolute horizontal position, some pipettes show a slight upward movement of the contained liquid. The upward displacement is due to the fact that adjustment to the upper mark was made in the verticle position against the hydrostatic pressure of the liquid in the pipette, whereas in the horizontal position this pressure no longer exists. However, if the pipette is held at a slight angle, 5 to 20 degrees to the horizontal, the liquid will not rise

above the mark, nor will it run out of the pipette when the vent is opened.) This all can be accomplished without in any way affecting the position of the meniscus within the micropipette.

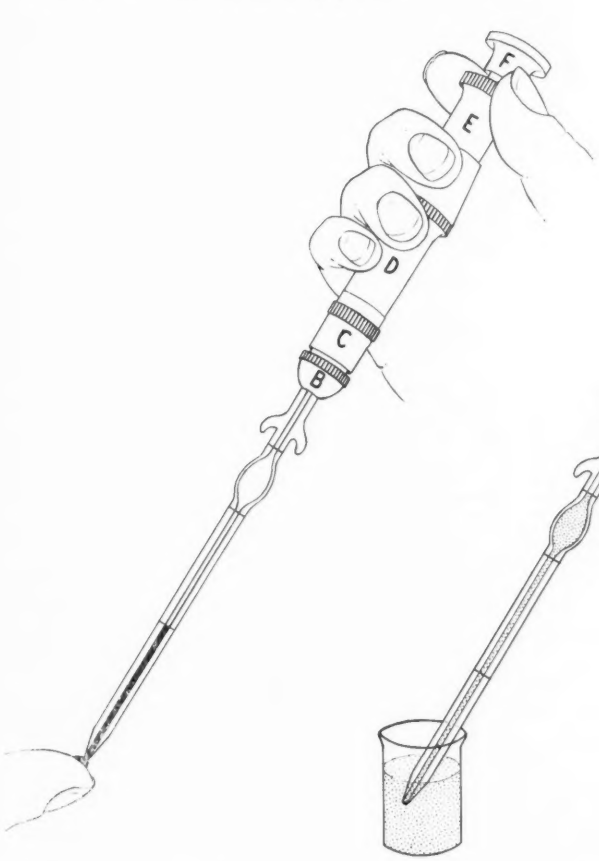

Fig. 3.

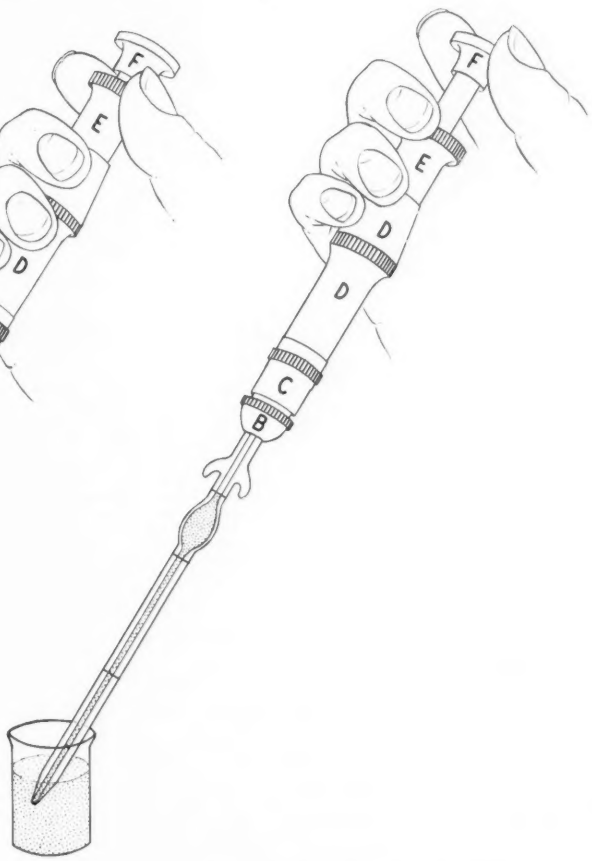

Fig. 4.

Fig. 3.—Measuring a drop of blood in the micropipette with the fine control screw (*E*).
Fig. 4.—Diluting the blood in the bulb of the pipette, using the plunger (*F*).

USE OF SCREW SYRINGE WITH 1 OR 2 C.C. PIPETTES FOR RADIOACTIVE OR OTHER TOXIC LIQUIDS

The pipette is inserted into the collet and the vent is closed. By means of the plunger *(F)* the liquid is sucked up into the pipette above the desired mark; the outside of the pipette may be wiped, if desired; the tip of the pipette is then placed against the side of the bottle, and the final adjustment to the mark is made with the screw device. The pipette is transferred to the desired container; the vent is opened, and the pipette allowed to drain by gravity.

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PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

Twenty-First Annual Meeting

Chicago, Ill., Oct. 29 and 30, 1948

ADDITIONAL ABSTRACTS—Concluded

69. THE CRYSTALLIZATION OF SEROTONIN

IRVINE H. PAGE, M.D., MAURICE M. RAPPORT, M.D. (BY INVITATION), AND ARDA ALDEN GREEN, M.D. (BY INVITATION)
CLEVELAND, OHIO

When organs are perfused with blood or serum, progressive vasoconstriction often makes it all but impossible to force blood through them. The substance causing this vasoconstriction has never been identified nor isolated. In view of its probable importance in the vasoconstriction occurring after rupture or thrombosis of vessels such as the coronaries, or after tissue injury in shock, it seemed important to find out something of its chemical nature.

The isolation from beef serum depended on (1) precipitation of beef serum proteins with alcohol, (2) precipitation of salts, phosphatides, and amino acids with acetone, (3) chloroform extraction, (4) extraction of the active principle with butyl alcohol, (5) precipitation of the active principle from butyl alcohol with 5-nitrobarbiturie acid, (6) decomposition by addition of acetone to hot aqueous solution, (7) extraction of filtrate residue with warm absolute methanol, (8) recrystallization from water-acetone.

Thin, rhomboid plates (m.p. 212-214°) were obtained which seem homogenous. Color reactions and ultraviolet absorption indicate the presence of an indole nucleus in the structure. The empiric formula is $C_{14}H_{23}O_7N_5S$. Ionic sulfate analysis suggests that Serotonin is a sulfuric acid salt of an organic base with the formula $C_{14}H_{21}O_3N_5.H_2SO_4$.

Serotonin is more than twice as active as adrenalin in causing constriction of the vessels of perfused rabbit ears. Isolated strips of rabbit ileum are contracted by it. Injected intravenously into dogs or cats it caused a sharp rise in arterial pressure much like that of adrenalin. The rise was augmented by sympathectomy. It does not appear to produce tachyphylaxis when given repeatedly in small doses.

An enzyme has been prepared from lung which inactivates Serotonin.

70. MANAGEMENT AND CLINICAL COURSE OF LOWER NEPHRON NEPHROSIS

WILLIAM S. HOFFMAN, M.D., AND DANIEL MARSHALL, M.D. (BY INVITATION)
CHICAGO, ILL.

Six patients with lower nephron nephrosis from a variety of causes have been managed by a regimen that included a deliberate, slow induction of edema of nearly normal electrolyte composition for the purpose of diluting the accumulating intoxication products. Other measures were designed to keep the patient in a good state of nutrition. The aim was to keep the patients from dying in uremia before the renal lesion began to subside. Five out of the six patients recovered, the sixth died in uremia and heart failure five days after the onset of diuresis. Four of the recovered patients had complete restoration

to normal renal function. One patient, a child of 2 years, still had moderate renal insufficiency five months after the onset of diuresis. This condition was associated with the development of bilateral calcinosis of the kidneys as seen on x-ray.

In these patients, the oliguric phase lasted from five to thirteen days. The serum nonprotein nitrogen rose to levels of 145 to 243 mg. per 100 c.c. and did not begin to subside until several days after diuresis began. Edema occurred early even when fluids were not specially administered. The serum chloride and sodium concentration tended to reach dangerously low levels (in one instance the chloride concentration was 58 meq. per liter). This development had to be combated by infusions of 2 per cent salt solution. With careful control of the quantity and composition of the edema fluid, symptoms of uremia were minimal, and the patients ate well without vomiting. The leveling of the serum nonprotein nitrogen concentration during the latter part of the oliguric period was due either to the diluting effect of the edema or to diminished tissue breakdown brought about by the improved nutrition.

71. STUDIES ON PROLONGED SUPPURATIVE INFECTION IN MAN

GEORGE W. JAMES, III, M.D., LILLIAN A. RIBLET, M.D., JOSEPH C. ROBINSON, M.D., ROBERT E. JOHNSON, M.D., AND ROBERT M. KARK, M.D., CHICAGO, ILL.

(INTRODUCED BY ROBERT W. KEETON, M.D.)

Despite advances in surgical techniques and the vigorous use of blood and plasma transfusions, sulfonamide derivatives, and antibiotic substances, suppuration persists in a number of patients with infected traumatic injuries. Therefore, during the past year clinical, bacteriologic, and laboratory studies were made on ninety-one young men suffering with chronic suppurating lesions of the bones, kidneys, and other soft tissues. A group of twenty-seven patients and ten bedridden control subjects were studied more intensively than the remaining sixty-four patients.

Although the average period of hospitalization was twenty-three months at the beginning of observation, fair nutritional status was maintained. There was an average weight loss of 7.9 kilograms but no clinical evidence of vitamin or protein deficiencies.

Sixty-three per cent of the patients were infected with *Staphylococcus aureus*. Pathogenic spore-bearing organisms, *Plocamobacterium aerogenes*, *Aerobacter aerogenes*, and gamma (nonhemolytic) streptococci were frequently isolated. Multiple infections and concurrent self-contamination were common findings, and a correlation was found between hemoglobin levels and numbers of different species of organisms in the wound. The more different genera of organisms, the lower the hemoglobin levels.

Hematologic data were essentially normal. Anemia was a rare finding. Blood and plasma volumes were increased 10 to 20 per cent when expressed as a function of body weight. Total circulating hemoglobin was significantly reduced in those patients with severe infections. A marked predominance of small spherocytic reticulocytes was observed, but no other aberrant cells were seen.

Depression in serum iron and elevation of serum copper levels were more marked in patients with severe infection. Serial studies during spontaneous remissions indicate an initial shift of serum copper levels toward normal. This is followed at a later date by elevation of serum iron levels. Preliminary observations following daily oral administration of 60 mg. cobalt chloride reveal a reticulocytosis during the third to fifth week of therapy and increases in red blood cell count, hemoglobin levels, hematocrit, and total circulating hemoglobin.

72. HAVERHILL FEVER (HAVERHILLIA MULTIFORMIS BACTEREMIA); THE BACTERIOLOGIC DIAGNOSIS

PRESTON E. HARRISON, M.D. (BY INVITATION), JAMES A. GREENE, M.D., AND ROBERT A. MORSE, M.D. (BY INVITATION), HOUSTON, TEXAS

The clinical manifestations of our cases of Haverhill fever have been reported previously. The present report is concerned with bacteriologic and serologic methods in the etiologic diagnosis of the disease.

Blood or joint fluid or other body fluids are cultured in brain-heart infusion broth to which 5 to 10 per cent blood plasma has been added and incubated under anaerobic conditions with 10 per cent carbon dioxide added. When growth is visible, usually after four to five days or as long as fourteen days, it is subcultured to a brain-heart infusion agar plate containing 10 per cent blood; the subculture plate is also incubated as outlined. The colony on the plate appears as a minute, round, clearly demarcated, and colorless growth, being almost invisible to the naked eye. Growth may be slow and sparse until several transfers have been made, when it may become more abundant. On a slide stained by Gram's method the organisms appear to stain poorly, and the bacterial cells appear pleomorphic. The predominating form in the primary blood culture and its subculture is a gram-negative, filamentous rod which varies considerably in size. These filamentous forms may appear to be branching, and some of them may contain irregularly staining granules, and their rods may demonstrate an irregular swelling along the course of the filamentous rods. In addition there may be many small, round coccus-like organisms, or coccoid rods, the so-called "L" bodies or pleuro-pneumonia-like organisms of Kleinberger. Serologic confirmation is made routinely by wet mount preparation containing known *Haverhillia multiformis* antiserum and a bacterial suspension from a typical colony suspected to be *Haverhillia multiformis*. Clumping of filamentous rods accompanied by capsular swelling of "L" bodies constitutes a positive test.

Diagnostic agglutination blood serum tests have been evaluated by using an antigen prepared as a saline suspension of a Merthiolate-killed organism from several strains of *Haverhillia multiformis*. The test has been performed in the usual manner and is comparable to the Widal test. A titer of 1:160 or higher is considered to be tentatively diagnostic. In cases with low titer agglutinin content, serial tests at weekly intervals may be helpful, since wide fluctuations are common; these fluctuations occur at various intervals in episodes of exacerbation of symptoms, or in periods of remission. The test does not appear to be entirely specific for *Haverhillia multiformis* infection. We have demonstrated agglutinins in high titer in apparently unrelated conditions such as tuberculosis and bronchogenic carcinoma of the lung. The significance of the nonspecific agglutinin test has not been evaluated thoroughly.

73. THE ROLE OF THE PLASMA PROTEIN FRACTIONS IN THE ACTION AND TRANSPORT OF PENICILLIN

R. E. DOLKART, M.D., B. HALPERN, B.S. (BY INVITATION), A. L. KUTZ, PH.D. (BY INVITATION), J. B. LESH, PH.D. (BY INVITATION), B. WOLNIAK, PH.D. (BY INVITATION), AND F. L. DEY, M.D. (BY INVITATION), CHICAGO, ILL.

Numerous reports have appeared dealing with the protein-binding effect and the interaction between penicillin and human plasma proteins. Some workers have concluded that penicillin combines with human serum albumin which

complex possesses antibiotic activity. Normal human subjects were given crystalline penicillin G following which a 500 ml. blood sample was fractionated into its various protein fractions. The penicillin was almost quantitatively recoverable in fraction VI, which in actuality represents that portion remaining after all other fractions have been precipitated. The plasma control assays for penicillin and the fraction VI assays were identical in each instance. Additional studies were carried out by dialysis of whole plasma and some of the various fractions under temperature conditions that would not be deleterious to penicillin. There was no evidence of inactivation or antagonism of the penicillin by any of the protein fractions nor evidence of any combination with the protein fractions in the dialysis experiments. Similar studies under way with bacitracin seem analogous.

74. THE ANESTHETIC AND ANTIHISTAMINIC ACTION OF A SERIES OF ANTIHISTAMINIC DRUGS IN HUMAN SKIN

JOHN U. KEATING, M.D. (BY INVITATION), AND CHARLES F. CODE, M.D.
ROCHESTER, MINN.

The anesthetic potency and the antihistaminic action of equimolar concentrations of Benadryl, Pyribenzamine, Neoantergan, 3015 R.P., and 3277 R.P. were determined in the skin of normal human subjects. In descending order of anesthetic potency, the drugs ranked: 3277 R.P., 3015 R.P., Pyribenzamine, Neoantergan, and Benadryl. In descending order of the ability of the drugs to inhibit histamine flares, they were: Pyribenzamine, Neoantergan, Benadryl, 3015 R.P., and 3277 R.P. The findings that those compounds which have the greatest local anesthetic potency are not those with the greatest antiflare effects supports the view that the flare-inhibiting property of these synthetic antihistaminic drugs is not directly dependent on their local anesthetic activity, and there is thus a separation between antihistaminic and anesthetic actions.

75. FAILURE OF ANTIHISTAMINIC DRUGS TO REDUCE REACTIVE HYPEREMIA IN MAN

MILTON LANDOWNE, M.D., AND WALTER S. THOMPSON, M.D. (BY INVITATION)
CHICAGO, ILL.

In the classic studies of Lewis and Grant on reactive hyperemia, evidence was presented suggesting that this vasodilatation was due to the accumulation of a chemical in the region of circulatory arrest. Barsoum and Smirk reported that a substance with the biologic properties of histamine could be detected in the venous blood during reactive hyperemia in concentrations up to .06 gamma per cubic centimeter. This evidence, together with the implication of Lewis' studies, has led to a tacit acceptance of the concept that reactive hyperemia is primarily due to the accumulation of histamine-like substance. The following observations, in which reactive hyperemia could not be reduced by antihistaminic drugs, seriously challenge the validity of this concept.

Reactive hyperemia was produced by the application and release of an inflatable occluding cuff placed about the thigh in seven subjects. These included normal subjects and one hypertensive patient before and after sympathectomy. Measurements of volume change and blood flow in the foot were made by means of a venous occlusion plethysmograph. Occlusions were maintained to produce manifest hyperemia (five to ten minutes) and threshold or minimal responses as well (one-half to three minutes). The initial inflow after release

of occlusion was considered to reflect the dilatation produced. After a period of control observations of resting flow and reactive hyperemia, Benadryl (beta-dimethylaminoethyl benzhydryl ether hydrochloride), 10 to 30 mg. intravenously, or Pyribenzamine (beta-dimethyl-aminoethyl-2-pyridyl-benzyl ammonium chloride), 50 mg. orally, was administered. Observations were repeated at intervals for a period of more than one hour. No diminution was detected in the circulatory response to arterial occlusion. The summarized averaged blood flows in cubic centimeters per minute per 100 c.c. limb volume, with the number of determinations in parentheses, are as follows.

	RESTING	REACTIVE HYPEREMIA
Before drug	2.70 (134)	8.33 (39)
After drug	2.62 (99)	8.78 (36)

There was no essential difference between different subjects or after varying durations of occlusions.

In several instances the intradermal injection of 0.1 to 1.0 gamma of histamin base demonstrated the effectiveness of the antihistaminic drug.

76. CLINICAL OBSERVATIONS ON HISTAMINE ADMINISTRATION DURING PREGNANCY

THOMAS W. MCELIN, M.D. (BY INVITATION), AND BAYARD T. HORTON, M.D.
ROCHESTER, MINN.

The recent obstetric, physiologic, and pharmacologic literature continues to discuss the oxytocic effect of histamine in women and in animals. As recently as 1946, editorial comment in the *Obstetrical and Gynecological Survey* reminded that histamine exerts a powerful stimulating effect directly on the myometrium. An impressive array of experimental literature may be found to document this opinion. Two of the earlier studies conducted were those of Hofbauer and of Bourne and Burn. The former author pointed out that uterine spasm is one of the observable responses in acute histamine poisoning in pregnant guinea pigs. Bourne and Burn in 1927, utilizing intrauterine bags with manometer attachments in parturient women, demonstrated that histamine injected subcutaneously in a dose of 2.0 mg. (reckoned in terms of base present) produced powerful but short-lived uterine contractions.

In the course of a prolonged program of clinical investigation and treatment of the so-called vascular diseases, allergic diseases, and certain atypical pain patterns not amenable to more usual forms of treatment, we have had occasion to administer or to prescribe the administration of histamine by the intravenous or subcutaneous route to fifteen pregnant women. In view of the apparent pharmacologic inconsistency of such therapy and because of the complete absence of any undesirable sequelae of such treatment, we wish to record our observations.

Our fifteen patients (twelve of whom had received a diagnosis of multiple sclerosis) were treated in a five-year period ending April, 1948. During this time approximately 70,000 intravenous injections of histamine were given and some 4,600 patients were seen in our laboratory. The series of pregnant patients included both primigravidas and multigravidas. Six patients received subcutaneous injections of histamine daily throughout the entire period of gestation. Intravenous injections of this drug were given in every month of pregnancy and in two instances the injections were given three times weekly throughout the entire third trimester to within three days of delivery. In thirteen of the fourteen patients who have thus far been delivered, there has been no

tendency to premature labor. Twelve of these fourteen have had their labor after the expected date of confinement.

The maximal amount of histamine given was administered to a primigravida, 23 years of age, who had multiple sclerosis. This patient received forty-five intravenous injections each of which contained 2.75 mg. of histamine disphosphate (1.0 mg. of histamine base) during the third, fourth, and fifth months of her pregnancy.

Only one event which might in any way be construed as representing an untoward effect on the pregnant uterus occurred in the entire series. A primigravida, 29 years of age, sustained one episode of slight vaginal bleeding twelve hours after a subcutaneous injection of 0.137 mg. of histamine diphosphate (0.05 mg. of histamine base). The month of the pregnancy in which this bleeding occurred is not known. This is one of the smallest amounts of histamine given to any patient in the group, so that the significance of this observation is questionable.

We wish to suggest on the basis of our experience that there is at least an apparent lack of a clinical oxytocic effect of histamine diphosphate when it is administered to pregnant women by the subcutaneous or intravenous route or by both routes in the therapeutic dosages mentioned. We offer no preferred explanation as to why the oxytocic effect described by so many authors and attested by abundant laboratory study did not occur. A few of the possible explanations which immediately present themselves are: (1) that the elevated histaminase level known to occur in pregnant women might be responsible for the inactivation of the injected histamine; (2) that the dosage of histamine used was not adequate to provoke a clinically observable oxytocic response; (3) that the altered neurogenic response in the twelve patients in this series who had multiple sclerosis may in some way, in these patients, have altered the predicted effect.

77. RETROBULBAR NEURITIS; TREATMENT WITH HISTAMINE

BAYARD T. HORTON, M.D., AND HENRY P. WAGENER, M.D. (BY INVITATION)
ROCHESTER, MINN.

WITH THE TECHNICAL ASSISTANCE OF EVELYN F. HELGERSON, M.A.

The typical syndrome of retrobulbar neuritis consists of lowered visual acuity, some form of scotoma in the visual field, and usually a normal-appearing nerve head. In both the acute and chronic forms of this syndrome, edema and interference of the blood supply to the optic nerve are evident. The axis-cylinders will withstand ischemia for considerable time before final degeneration begins. A vasodilating agent which will increase materially the blood supply to the optic nerve is the most satisfactory means of restoring its function. Spontaneous recovery may and frequently does occur, but in our experience, unless it begins within three weeks after the onset of symptoms, it will not be complete.

Histamine is the most powerful vasodilating agent available for increasing the blood supply to the central nervous system; hence the rationale for its use in the treatment of retrobulbar neuritis. Compared with other forms of treatment, it is more universally applicable, is less discommoding to the patient, does not necessitate hospitalization, and seems to result in more rapid and complete recovery of vision.

In this study we have employed a 1:250,000 dilution of histamine administered intravenously by the drip method at rates varying from 24 to 48 drops

per minute. The rate employed depended on the patient's tolerance. Treatment for one and one-half hours has been carried out daily or every other day for from one week to three and one-half years. No untoward reactions have been observed. One patient received 410 treatments. In the light of our recent experiences it is obvious that some of our earlier patients had inadequate treatment.

During the past six years we have administered histamine to sixty-one patients who had retrobulbar neuritis. Of these sixty-one patients, thirty-four were women and twenty-seven were men. The ages ranged from 9 to 49 years, with an average age of 30.5 years.

Eighteen patients previously had been given typhoid vaccine intravenously. None of the eighteen regained normal vision, seven obtained 25 to 75 per cent improvement in vision, ten noted no change, and one experienced greater loss of vision after treatment with typhoid vaccine. When these same eighteen patients were treated with histamine administered intravenously, five regained normal vision, five obtained 25 to 75 per cent improvement, and eight noted no change.

Forty-three of the sixty-one patients had not had previous treatment with typhoid vaccine but did receive histamine intravenously. Of these forty-three patients, nineteen regained normal vision; three obtained 75 per cent recovery; six, 50 per cent recovery; five, less than 25 per cent recovery; and the remaining ten noted no change.

Twenty-two of the sixty-one patients had had visual loss for more than one year; five of these regained normal vision. Five of the sixty-one patients had had visual loss for six months to one year and none of these regained normal vision. Eight of the sixty-one had had visual loss for three to six months and six of these regained normal vision. Twenty-one of the sixty-one patients had had visual loss for one month or less; twelve of these regained normal vision. Five of the sixty-one patients were unable to furnish definite data as to the onset of their visual loss and of this group one obtained normal vision.

In summary, it is interesting to note that twenty-four of the sixty-one patients regained normal vision and that the vision of ten improved 50 to 75 per cent. Although eighteen of the sixty-one patients had had previous treatment with typhoid vaccine, none of the group had normal vision following this type of treatment. However, when histamine was administered intravenously to this same group of patients, five regained normal vision.

78. THE PREPARATION AND PROPERTIES OF MAMMALIAN STRIATED MYOFIBRILS

ARMIN F. SCHICK, M.D. (BY INVITATION), AND GEORGE M. HASS, M.D.
CHICAGO, ILL.

A physicochemical method for isolation and purification of myofibrils from mammalian skeletal and cardiac muscle has been devised. Blocks of living fresh muscle were frozen and cut into thin sections with a freezing microtome. The sections were transferred directly to a proteolytic enzyme buffer solution (0° C.; pH, 7; ionic strength, 0.25). After digestion at 0° C. for thirty to forty-five minutes, the myofibrils were separated by mild mechanical agitation. Following this, the segregated myofibrils were separated from other cellular and interstitial components of muscle by controlled centrifugation.

The isolated myofibrils which were obtained in large quantities by this method had characteristic physical properties. They varied in length and

breadth but retained the essential microscopic structural detail, plasticity and birefringence, characteristic of myofibrils in their normal intracellular location, prior to application of the procedure for isolation. They were soluble in a slightly alkaline reagent (0.5 normal potassium chloride plus 0.03 normal sodium bicarbonate) giving viscous solutions which displayed a strong birefringence of flow. In buffer solutions with an ionic strength of 0.15, the myofibrils dissolved on the acid side of pH 4.0 and on the alkaline side of pH 10.0. In phosphate buffer solutions with an ionic strength of 0.5, the myofibrils dissolved when solutions were on the alkaline side of pH 6.3. In phosphate buffer solutions with the ionic strength increased from 0.15 to 0.5 by addition of potassium chloride, the myofibrils were soluble when solutions were on the alkaline side of pH 6.0.

The isolated myofibrils, although probably modified in some respects by tryptic action and mechanical agitation during isolation at 0° C., exhibited the property of contractility when placed in dilute neutral solutions of adenosine triphosphate. Under these conditions, myofibrils isolated from skeletal muscle of man and rabbit contracted rapidly so that long fibrillar structures with sharply defined microscopic characteristics were converted irreversibly into spherical masses with no recognizable structural detail.

79. CASTOR BEAN SENSITIVITY

W. P. Garver, M.D., Cleveland, Ohio

Five men working in a mill where castor bean is processed complained of allergic symptoms associated with this occupation.

The five were skin tested with seven extracts of castor bean products. All of them had a positive reaction to one or more of the extracts. Three of the five were then tested by the Prausnitz-Küstner passive transfer technique. The reagins identified by this method were identical with the positive tests obtained by direct skin testing. An extract of castor pomace gave a positive test in all five patients. All of the patients associated contact with this substance with their clinical symptoms.

Thirteen control subjects were skin tested with the same extracts. Eight failed to react to any of the castor extracts. Three gave slight irritative nonspecific reactions. Two control subjects reacted in some degree to most of the extracts. The latter two were foundrymen suffering from asthma. The relationship of these positive tests to their asthma has not been determined.

80. BERYLLIOSIS: OBSERVATIONS AND REPORT OF CLINICAL STUDY OF SEVENTY CASES OF CHRONIC DISEASE

Willard Machle, M.D., New York, N. Y.

Berylliosis may be defined as a general disease characterized chiefly by pulmonary insufficiency and having the major pathologic changes in the lung. It results from the inhalation of finely divided beryllium compounds.

The epidemiology and etiology of the disease are discussed, and a summary of the clinical study of seventy cases of chronic disease is presented. Report is made upon chemical findings in the tissues of persons with fatal cases and the pathologic anatomy of the disease is discussed briefly. Data on concentrations of beryllium in the air of plants are reviewed. X-ray films from representative cases will be presented.

81. CORRELATION OF LACTOBACILLUS COUNTS WITH EXTENT OF DENTAL CARIES IN AN INSTITUTIONAL POPULATION

JULIAN D. BOYD, M.D., V. D. CHEYNE, D.D.S. (BY INVITATION), AND K. E. WESSELS, D.D.S. (BY INVITATION), IOWA CITY, IOWA

Lactobacillus counts from the saliva of more than 200 institutionalized children have been made in a State Laboratory by personnel especially trained to provide such service to the dentists of the state. Duplicate analyses of samples collected within twenty-four to forty-eight hours were made for most of the subjects. All samples were collected before breakfast and before cleaning of the teeth after the subjects chewed paraffin.

The individual lactobacillus counts were correlated with the extent of tooth decay (number of DMF surfaces), the rate of progression of tooth decay during the previous nine to twenty-one months, and the status of mouth hygiene. No correlation was evident except with the habitual and the current state of oral hygiene. The data offer nothing to support the premise that lactobacillus counts provide a diagnostic or prognostic index of the activity of dental caries. Any apparent relationship evidently relates to the hygiene of the oral cavity and the areas for lodgment of pabulum for bacterial growth rather than to the process of caries in itself.

82. ACUTE TORTICOLLIS DUE TO FOOD ALLERGY

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

Repeated attacks of acute torticollis have been observed to follow the ingestion of allergenic foods in each of three specifically sensitized patients.

Although the symptoms of taughtness, pulling, aching, and tenderness of the posterior cervical muscles, with and without associated headaches, have been observed as manifestations of chronic food allergy and such symptoms have been induced following the experimental ingestion of allergenic foods, instances of acute torticollis have not been described on this basis. As a rule, recurrences of cervical myalgia on an allergic basis do not go on to the development of acute torticollis.

The most favorable circumstances for the development of an allergic wry neck include the presence of a high degree of specific sensitivity to a commonly ingested food which previously had been avoided for at least three or four days prior to an evening feeding of this food. This, in turn, is usually associated with an allergic reaction of such severity as to cause the patient to retire shortly after the evening meal. Motion on turning in bed during sleep, upon arising from bed the following morning, or within the first half hour after arising may be associated with the sudden onset of an excruciatingly severe pain sharply localized to a small exquisitely tender area in the trapezius or sternocleidomastoid muscles. Contracture of the involved muscles with immobilization of the head in a position favoring the shortening of the affected muscle or muscle groups immediately results as a protective measure because of the extreme pain on motion of the head. A relatively fixed position of the head and shoulders is maintained for one or two days following which the attack gradually subsides coincident with a decrease in the hypertonicity, aching, pain, and tenderness of the affected muscles.

83. THE EFFECT OF VARYING THE DOSE OF IODINE ON THE BEHAVIOR OF RADIOIODINE TRACERS IN PATIENTS WITH EXOPHTHALMIC GOITER

D. S. Childs, Jr., M.D. (By Invitation), F. R. Keating, Jr., M.D., M. M. D. Williams, Ph.D. (By Invitation), and M. H. Power, Ph.D.
Rochester, Minn.

Five patients who had exophthalmic goiter received repeated tracer doses of I^{131} by mouth, with varying quantities of stable sodium iodide as carrier. The behavior of each dose was followed by means of serial measurements of the intensity of radiation over the thyroid gland and abdomen, and of the concentration of I^{131} in blood serum and urine.

Where small quantities of iodine (1 to 100 μg) were used, a large proportion of the dose was accumulated and retained in the thyroid gland. The quantity of I^{131} in the thyroid gland increased exponentially for six to twenty-four hours, and thereafter decreased very slowly.

When large quantities of iodide (10 mg.) were employed, little or none of the dose remained in the thyroid gland and a large proportion (80 per cent) appeared in the urine. Observations over the thyroid gland revealed a prompt but temporary accumulation in the thyroid gland which reached a peak representing 15 to 35 per cent of the dose within three hours. Subsequently, the radioiodine content of the thyroid gland decerased rapidly until at twenty-four hours only a small fraction remained. This decrease of I^{131} in the thyroid gland occurred at a rate comparable to, but somewhat slower than, the rate of disappearance of radioiodine from the blood.

When an intermediate quantity of iodide (1.0 mg.) was used, the curves describing the changes in concentration of I^{131} in the thyroid gland appeared to be composites of those obtained in the high and low carrier groups. The rate of disappearance of radioiodine from the blood and the quantities eventually accumulated in the thyroid gland or excreted in the urine likewise were intermediate between the other groups.

It is suggested that the iodine-accumulating function of the hyperactive thyroid gland observed after the use of small doses of labeled iodide is largely or entirely related to the synthesis and storage in the thyroid gland of organic compounds containing iodine.

The function of the hyperactive thyroid gland which is observed when large doses of iodide are administered appears to be similar to that described by Astwood in the normal human thyroid gland blocked with antithyroid drugs, and appears to represent the storage within the thyroid gland of iodide as such.

The function of the kidney with respect to the excretion of iodide did not appear to be materially altered over the range of doses herein employed.

84. EVALUATION OF THE USE OF ANTERIOR PITUITARY EXTRACT IN THE TREATMENT OF PITUITARY DWARFISM

Joseph C. Edwards, M.D., Cecil M. Charles, M.D. (By Invitation), and Cyril M. MacBryde, M.D., St. Louis, Mo.

This study was based on the treatment of eleven dwarfs with pituitary growth extract. Eight patients exhibited all of the characteristics of pituitary dwarfs. Case 4 had had satisfactory treatment for congenital syphilis. A twelfth dwarfed patient with normal epiphyses and genital development reached normal size in two years with treatment by desiccated thyroid, 0.06 Gm. daily

alone. Case 6 had had Pott's disease of the tenth dorsal vertebra. Cases 3, 6, and 7, although having the appearance of pituitary dwarfs with genital and general physical underdevelopment, had no delay in epiphyseal development for their ages.

Method of Study.—After preliminary periods of observation with accurate anthropometric measurements, each of the eleven patients was given Phyone pituitary extract (Wilson), which contains both growth and thyrotropic factors. An initial dose of 0.25 c.c., then doses of 0.5 and 1.0 c.c. on alternate days were given intramuscularly. A 2.0 c.c. dose thrice weekly was continued for two to six months with control intervals without treatment.

Basal metabolic rates were normal in all but three patients. No patient had myxedema. Cases 1, 4, and 8 received daily doses of 0.03 to 0.12 Gm. of desiccated thyroid daily in addition to Phyone treatment because of slightly subnormal basal metabolic rates. Case 11 received thyroid a short time to note any added effect on growth; no significant change was noted. The other eight patients receiving Phyone were not treated with any thyroid, androgen, or estrogens during their observation on Phyone treatment.

Blood counts, hemoglobin, urine analyses, and blood Kahn tests were normal. Roentgenograms of the skull were normal in nine patients, and two had bridging of the clinoids with small sella turcicas.

Results.—Case 3 was the only one of the eleven whose height and weight at the end of treatment exceeded the minimum average height and weight for his age. The normal growth curves for boys and girls are shown in the same scale as the growth curves of this group for comparison.

Our patients failed to show an increase in stature or weight so definite that it could be attributed to the use of the growth extract Phyone alone.

Conclusion.—Further studies with more potent pituitary growth hormones are needed before one can unreservedly recommend the use of commercial growth hormones for prolonged treatment of pituitary dwarfism.

85. EFFECT OF INSULIN ON TUBULAR REABSORPTION OF GLUCOSE IN DIABETIC PATIENTS

MELVIN M. CHERTACK, M.D. (BY INVITATION), Luke J. GRIMELLI, M.D. (BY INVITATION), HELEN L. RHETTA, M.D. (BY INVITATION), AND ROBERT W. KEETON, M.D., CHICAGO, ILL.

In 1941 Shannon and associates reported a 10 per cent reduction of the renal tubular reabsorption of glucose (TM_G) in four out of six dogs following an injection of 50 units of insulin. Creatinine was used to measure glomerular filtration. The ratios of TM_G subsequent to insulin divided by their values prior to insulin were 0.88, 0.89, 0.87, 1.06, 0.77, and 0.96.

In our studies five adult male patients, ages 18 to 33, known to be diabetic for one to eight years, were used. They showed no clinical or laboratory evidence of hypertension, renal disease, or other complications. They were readily desugarized on insulin and were classified as insulin sensitive. Insulin dosages varied from 10 to 35 units of crystalline and from 20 to 50 units of protamine.

The patients were stabilized on diet and insulin. On the morning of the insulin experiment the daily doses of protamine and crystalline insulins were given; the glycemic level was raised; and the TM_G values were determined through three periods. Several days later after the protamine insulin had been withheld for not less than forty-eight hours and crystalline insulin for twenty-four hours, the experiment was repeated. The ratios of TM_G after the insulin

divided by their values without insulin were 0.98, 0.92, 0.83, 0.83, and 0.77, respectively, for the five subjects. It has been generally recognized that variation from period to period in TM_G determinations may be large.

The plan used by other workers of averaging the values for three successive periods to secure the TM_G of the patient was followed. Although these values were depressed consistently after the administration of insulin, the degree of depression was not regarded as significant in view of the limitations of the method.

It should be realized that all of the patients had an endogenous supply of insulin in addition to the injected supply. One may, therefore, conclude only that a deficiency in insulin does not interfere significantly with tubular reabsorption of glucose.

86. EFFECT OF HYPERGLYCEMIA ON THE CLEARANCES OF INULIN AND PARA-AMINOHIPPURIC ACID

L. J. Grimelli, M.D., M. M. Chertack, M.D., H. L. Rhetta, M.D., A. B. Kendrick, Ph.D., and R. A. Forrest, M.D., Chicago, Ill.

(Introduced by Robert W. Keeton, M.D.)

Early in our studies of renal function in the presence of hyperglycemia, certain discrepancies in the determination of PAH clearance were noted. Klopp, Young, and Taylor reported that hyperglycemia may decrease the PAH clearance from 30 to 80 per cent but not effect the clearance of mannitol. It seemed wise to study this problem further.

In the present studies to be reported, insulin was maintained at levels of 20 to 75 mg. per cent, the para-aminohippuric acid (PAH) at 0.9 to 3.5 mg. per cent, and the glucose at normal fasting to hyperglycemic levels.

Thirteen patients, of whom seven were women and six were men, were studied. The previous clinical data had shown normal kidney function. The experiments were done in the following manner: (1) at normal glycemia and low levels of PAH; (2) at hyperglycemia and low levels of PAH; and (3) at hyperglycemia and high levels of PAH. The inulin clearance rose slightly but progressively under the experimental conditions enumerated. This rise was not significant since it fell within the limits of the standard deviation of the method. All values were corrected to a surface area of 1.73 square meters. At fasting blood sugar levels the PAH_c in women averaged 593.8 ± 126.3 c.c. per minute, and at hyperglycemia, 407.1 ± 146.1 c.c. per minute. The average depression due to hyperglycemia was 31.4 per cent.

In the men the PAH_c at fasting glucose levels averaged 654.9 ± 105.3 c.c. per minute, and at hyperglycemia, 312.5 ± 119.5 c.c. per minute, with an average depression of 52.5 per cent. The depression of PAH_c was reproduced on separate days in one patient. At fasting glucose levels the TM_{PAH} was 76.9 mg. per minute, and at hyperglycemic levels the TM_{PAH} was 54.0 mg. per minute. The average depression due to hyperglycemia was 29.8 per cent.

Hyperglycemia depresses the PAH_c and the TM_{PAH} values. Sufficient experiments have not been conducted to establish correction factors. The competitive antagonism between glucose and para-aminohippuric acid as it affects tubular function is now under investigation.

87. CHOLESTEROL TOLERANCE TESTS IN NORMAL, DIABETIC, AND HYPERTENSIVE PATIENTS

E. D. Futch, M.D., and Raymond Gregory, M. D., Galveston, Texas

The possibility that intimal atherosclerosis is due in some instances to a basic metabolic fault in the capacity to utilize cholesterol caused us to devise a cholesterol tolerance test which will be briefly described.

This test was performed on fourteen normal subjects; four patients with essential hypertension; eleven patients with diabetes mellitus, 23 to 79 years of age; one patient with hypercholesterolemia and xanthomatosis of the skin; one patient with nephrotic stage of chronic glomerulonephritis and hypercholesterolemia; one male patient, 25 years of age, with acute myocardial infarction and normal serum cholesterol.

Repeated analyses established that the method entailed a maximal error of 5 per cent. In the group of fourteen normal subjects, the total free cholesterol increased from 4 to 38 per cent; cholesterol ester increased 0.8 to 24 per cent. Maximal increases were distributed from the second to the eighth hour. In two subjects the free and ester forms fell 5 to 18 per cent.

The results in the eleven diabetic patients were the same as in the normal subjects in all essential respects. The results in four patients with essential hypertension likewise were within the range shown by normal subjects. In the patient with skin xanthomas and in the patient with nephrosis, both of whom had hypercholesterolemia, the blood levels of both free and ester forms of cholesterol fell 3 to 19 per cent during the tolerance test.

The only abnormal results were found in the 25-year-old man with acute myocardial infarction. In his case the free cholesterol rose 43 per cent and the cholesterol esters rose 29 per cent. The maximal rise in free cholesterol in the normal group was 38 per cent in one instance, with an average maximal rise of 17 per cent. The maximal rise in cholesterol ester in the normal group was 24 per cent, with the average maximal rise of 9 per cent.

These data do not demonstrate any difference between normal, hypertensive, and diabetic subjects in their response to a cholesterol tolerance test. This indicates that the tendency of the diabetic and hypertensive patient to develop intimal atherosclerosis is not due to a prolonged or excessive postprandial hypercholesterolemia.

88. THE MEDICAL AND METABOLIC FACTORS IN THE SURGICAL MANAGEMENT OF HYPERTHYROIDISM

I. Darin Puppel, M.D., Columbus, Ohio

The goitrogens are by no means solely responsible for the decreased morbidity and mortality following the modern thyroidectomy. There are a multitude of factors which must still be considered in the proper surgical treatment of the patient with hyperthyroidism in addition to the use of the goitrogens.

A summary of consecutive thyroidectomies on 400 goitrous patients from 1937 to 1942, and prior to use of our present regimen, revealed 185 to be for nontoxic goiter and there resulted no hospital deaths. Two hundred fifteen were for hyperthyroidism and these accounted for an unusually high degree of morbidity and mortality so that the total mortality averaged 2.25 per cent. During this same period four pole ligations were done and ten thyroidectomies were staged, followed by a high mortality. The causes of death included thyroid crisis, respiratory failure, massive atelectasis, bronchopneumonia, acute cardiac failure or shock, and liver death. These represent to a great extent avoidable

causes of death in the light of our present methods of treatment. Consequently, a summary of consecutive thyroidectomies on 400 goitrous patients treated by our present regimen from 1943 to 1948 revealed 157 to be for nontoxic goiter, and there occurred no deaths. Two hundred forty-three were for hyperthyroidism which is a greater number than in the former group. Furthermore, the severity of the hyperthyroidism in this group was usually greater and the complications were more frequent. Yet there resulted no deaths. No ligations were done and only five thyroidectomies were staged, but with no deaths.

In our experience, there are two groups of medical and metabolic factors which have led to this lowered morbidity and mortality rate of the modern operation. The first includes seven general factors and major developments which have made all modern surgery less hazardous. The second group embraces at least nine specific factors related to hyperthyroidism which have counteracted the deleterious hypermetabolic effects of the disease.

The clinical use of the goitrogens and internal radiotherapy is still in many ways in the experimental stage. When a physician contemplates the use of one of these substances he should seriously consider the general and specific factors in the treatment of toxic goiter in addition to the new substance in order to serve the best interests of the patient.

89. THE BETA GLUCURONIDASE ACTIVITY OF HUMAN ENDOMETRIUM

LESTER D. ODELL, M.D., AND WILLIAM H. FISHMAN, PH.D. (BY INVITATION)
CHICAGO, ILL.

Endometrial biopsies (suction curette) were obtained from thirty-two women at various days of the normal menstrual cycle. The specimens were weighed, homogenized, and assayed for β-glucuronidase activity using the method of Fishman, Springer, and Brunetti (J. Biol. Chem. **173**: 449-456, 1948).

The β-glucuronidase activity increased during the first two-thirds of the menstrual cycle, followed by a decline, particularly in the last three days of the cycle. The presence or absence of progestational endometrium seemed to bear little reference to these changes. These results would indicate that the β-glucuronidase activity of endometrium parallels the estrogen secretion reported for the menstrual cycle.

Multiple specimens of endometrium were obtained from the same patients and assayed independently. While there was some variation in results, it was insufficient to disturb the general pattern described here.

β-glucuronidase is believed to play a fundamental role in the physiologic action of the estrogenic hormones. The cyclic change in activity observed in the endometrium parallels the histologic effect of estrogen stimulation. Therefore, it is possible that endometrium normally participates in the metabolic conjugation of estrogenic hormones.

90. DIFFUSE PANCREATIC CALCIFICATION WITH DIABETES MELLITUS

BRUNO J. PETERS, M.D., M. C. F. LINDERT, M.D., MARTIN J. KLAPMAN, M.D., AND DANIEL J. MENDELSON, M.D., MILWAUKEE, WIS.

(INTRODUCED BY MAURICE HARDGROVE, M.D.)

The present study is one of the association of pancreatic calcification with diabetes mellitus. In addition to four cases observed clinically, there are three cases of pancreatic calcification from which autopsy findings are presented. It

has been suggested that disseminated pancreatic calcification be recognized as a distinct clinical entity. We feel that it is impossible to differentiate clinically disseminated calcification from multiple calculi in the pancreas. The etology of the change is unknown. Alcoholism was the one most frequent, suspicious, predisposing cause in our cases. The symptoms simulated those of gall bladder colic, perforated ulcer, and appendicitis. It is suggested that a scout film be taken in acute abdominal conditions generally and in a diabetic patient particularly. In this way more cases will be recognized and erroneous surgery will be prevented. Also, with the advent of recent advances in pancreatic surgery, it is conceivable that partial or total pancreatectomy may be helpful in relieving the intractable pain present in some of the patients.

91. PSEUDOHYPOPARATHYROIDISM

Case Report

M. G. Peterman, M.D., and J. L. Garvey, M.D. (By Invitation)
Milwaukee, Wis.

A 12-year-old girl was examined because of logorrhea, mental retardation, and convulsions. The patient's condition had been diagnosed as epilepsy and hypothyroidism. The mother was ill during pregnancy and almost miscarried in the fourth month. Delivery and infancy were normal. The infant was always hungry, never lost infantile fatness, always had a hoarse voice, and often a crowing inspiration. At 2 years she complained of numbness and tingling in the extremities.

The patient weighed 101 pounds and her height was 58½ inches. She had a round, pudgy face with a slightly yellow pallor of the skin and a generalized myxedematous appearance. Chvostek's sign was strongly positive as were the ulnar and peroneal reflexes. The Trousseau test was negative. The voice was hoarse and coarse. There were several areas of calcification in the skin. The mouth was kept in a "carp" position.

The girl had repeated episodes of sudden squealing or shouting with pupils dilated and without loss of equilibrium. The mouth was held open; the extremities were tense. There were five to fifteen seizures in each twenty-four hours.

The serum calcium was 6.6; phosphorus, 11 milligrams. The urinary phosphorus excretion was 0.7 Gm. in twenty-four hours. The Sulkowitch calcium test of the urine was negative throughout.

Lateral x-ray of the skull showed scattered areas of calcification in the basal nuclei and generalized osteoporosis. This osteoporosis was also found in all of the long bones.

The electrocardiogram showed a marked increase in the Q-T interval.

$$K = \frac{Q\text{-}T}{RR} = .51.$$

The electroencephalogram showed spiking, slow 6 per second waves, a big build-up on hyperventilation with 3 per second waves not typical of the dysrhythmia of epilepsy.

The Binet-Simon test gave an I. Q. of 79; the Rorschach test: "there is much in the structure of the record and in the child's behavior during the test to indicate an organic brain disturbance of an epileptic or convulsive type."

An intravenous pyelogram showed normal kidneys and ureters. The Mosenthal test was negative.

There has been a good response to treatment with high-calcium, low-phosphorus, acid-ash diet. Treatment with parathyroid extract was ineffective. Treatment with acid-ash, high-calcium, low-phosphorus diet plus Hytakerol brought the blood chemistry to normal. However, the seizures have persisted.

No case of hypoparathyroidism or of the pseudo-type has been reported with osteoporosis of the bones.

92. PREMATURE CALCIFICATION OF THE COSTAL CARTILAGES: ITS FREQUENT ASSOCIATION WITH PSYCHONEUROSES AND POSSIBLE ENDOCRINE IMBALANCE

JOHN L. HORNER, M.D., ST. LOUIS, MO.

(INTRODUCED BY S. B. GRANT, M.D.)

Calcification of the costal cartilages is a process which, while presumed to be a matter of ageing, is frequently found in young persons.

It is generally considered that this deposit is a matter of no consequence. For this reason, perhaps, very little progress has been made in determining the factors which cause, or are associated with, the calcification. Association with various organic diseases, such as pulmonary tuberculosis, has been postulated but disproved.

A study of 277 consecutive patients under the age of 40 years has been made. One hundred fifty-eight of these were shown by x-ray to have costal cartilage calcification, while 119 did not. In addition to age and sex, the following details from the case history were noted: the complaints, the state of nutrition, the basal metabolic rate, the blood calcium, history of menstrual disorders in the women and the final chief diagnosis.

Significant differences between those persons who showed calcium in the costal cartilages and those who did not were found in the number of chief complaints, the history of menstrual disorders, the percentage with obesity, and the final chief diagnosis. In the former group there were twice the number of complaints, twice the incidence of obesity, and three times the incidence of menstrual disorders. Most remarkable was the fact that the complaints were *not* due to organic disease five times as often in the calcification group.

It is suggested that premature calcification in the costal cartilages may be evidence of a link between psychiatric and previously unrecognized endocrine disturbances. Further experimental work on young persons showing this phenomenon is needed.

93. PSYCHOSOMATIC PROBLEMS IN A PRIVATE PRACTICE

EDWARD J. RYAN, M.D., AND PHILIP W. MORGAN, M.D. (BY INVITATION)
EMPORIA, KAN.

This analysis was made from the office records of two internists in private practice in a community of 15,000 which has a large agricultural drawing area. Heavy industry, with its attendant physical and psychological problems, is not a factor.

To determine the incidence of functional problems, 300 consecutive complete office studies were analyzed. Each patient in the study had been subjected to detailed history, complete physical examination, and a minimum laboratory and roentgen survey consisting of urine analysis, blood count, sedimenta-

tion rate, and chest fluoroscopy, with chest film if considered necessary. Additional appropriate laboratory or other procedures had been utilized where indicated.

It is fully realized that an analysis of this type cannot be entirely objective, and that the personal equation must enter into the classification of certain patients. As nearly as possible, those patients with demonstrable organic disease which might explain the symptoms were eliminated from the functional or psychosomatic classification. Essential hypertension, peptic ulcer, and urticaria were classified as organic disease in this survey.

The 300 cases comprised 119 men and 181 women.

Of the 300 cases, ninety-two, or 31 per cent, were considered to fall within the functional or psychosomatic classification. These ninety-two cases comprised thirty men and sixty-two women. On the basis of these figures, 25 per cent of all the men studied and 34 per cent of all the women studied could be so classified. Men constituted 40 per cent of the total series and 33 per cent of the functional problems.

An attempt was made to classify the primary complaint of each of these ninety-two patients within an organ system:

SYSTEM	CASES
Circulatory	27
Respiratory	3
Gastrointestinal	21
Urinary	2
Neuromuscular	6
Endocrine	1
Visual	1
Oral (buccal lesions)	1
General (fatigue, tension, headache)	30

A separation of the three major classifications according to sex is as follows:

SYSTEM	TOTAL	MEN	WOMEN
Circulatory	27	12	15
Gastrointestinal	21	5	16
General (fatigue, etc.)	30	9	21

The oldest woman in the functional series was 69 years of age, the youngest, 13. Fifty-five per cent were under 40 years of age which would appear to minimize the climacteric factor in production of symptoms. The oldest man was 67 and the youngest 21.

Analysis of causative factors was difficult. Anxiety, in one guise or another, appeared to be of major importance.

Beneficial results of management coincided closely with the time, patience, and practical common sense devoted to each case.

94. THE EFFECT OF ENTEROGASTRONE CONCENTRATES ON GASTRIC SECRETION IN HUMAN BEINGS

CARL G. MORLOCK, M.D. (BY INVITATION), RICHARD R. FERAYORNI, M.D. (BY INVITATION), AND CHARLES F. CODE, M.D., ROCHESTER, MINN.

We have studied the effect of enterogastrone concentrate on the gastric secretory response of human beings to the injection of histamine and to the ingestion of a test meal. An enterogastrone concentrate prepared for parenteral administration and one prepared for oral administration were used in this study. The activity of the material prepared for parenteral administration was tested on rats and dogs before it was used in human volunteers.

The enterogastrone preparation given parenterally produced a threefold to fourfold reduction in the volume of gastric juice secreted by rats after pyloric ligation. When the enterogastrone was given intravenously in doses of 100 mg. or more to dogs with Heidenhain pouches secreting in response to histamine, pronounced inhibition of acid output uniformly occurred.

Enterogastrone concentrate given intramuscularly to ten human volunteers in doses of 200 mg. did not affect significantly the gastric secretory response to the injection of histamine during a double histamine test. Enterogastrone concentrate given to fourteen human volunteers in doses as large as 400 mg. intramuscularly and 18 Gm. orally did not significantly affect the gastric secretory response to a modified Ewald test meal.

Although the extract of hog's intestinal mucosa used for parenteral administration inhibited gastric secretion in rats and dogs, the immediate conclusion that this was due to enterogastrone was tempered by the fact that toxic reactions sometimes accompanied its use in dogs. Because contaminating substances may have prevented action by enterogastrone in human beings, we do not feel that our results can at this time be interpreted to indicate that pure enterogastrone will be without action in similar tests on human beings.

95. A COMPARISON OF THE TWELVE-HOUR NOCTURNAL GASTRIC SECRETION IN UNCOMPLICATED DUODENAL ULCER BEFORE AND AFTER HEALING

ERWIN LEVIN, M.D. (BY INVITATION), JOSEPH B. KIRSNER, M.D., AND WALTER LINCOLN PALMER, M.D., CHICAGO, ILL.

The twelve-hour nocturnal gastric secretion was measured in thirteen patients with duodenal ulcer during a period when the ulcer was easily demonstrable roentgenologically and when typical distress was present. The studies were repeated in the same individuals after medical treatment had led to healing of the ulcer roentgenologically and to the complete subsidence of symptoms.

During the period of active ulcer, the volume for the entire group averaged 1,047 c.c., the free acidity of the total volume averaged 58 clinical units, and the average output of acid 2,208 milligrams. After healing of the ulcer, there was no significant change in the average nocturnal gastric secretion, the volume averaging 1,002 c.c., the free acidity 54 clinical units, and the output of acid 1,957 milligrams. A significant decrease after healing was noted in the volume in one patient, in the concentration of acid in two, and in the output of acid in one individual. An increase in gastric secretion after healing was observed in a similar number of cases.

The average nocturnal gastric secretion in patients with healed duodenal ulcer is significantly greater than that found in a group of thirty-three healthy normal individuals studied under identical conditions. The secretion of acid in all the patients was continuous and was maintained at a relatively higher level than is seen in normal individuals. In no instance were there periods of anacidity for as long as one hour such as are frequently encountered in normal persons.

The persistence of hypersecretion in the vast majority of cases after healing of duodenal ulcer emphasizes the importance of continued careful antacid therapy in such patients.

96. THE INCIDENCE OF SYMPTOMS AND THE GASTRIC SECRETORY RESPONSE TO HISTAMINE IN PATIENTS WITH AND WITHOUT CHRONIC GASTRITIS

JOHN W. FINDLEY, JR., M.D. (BY INVITATION), JOSEPH B. KIRSNER, M.D., AND WALTER LINCOLN PALMER, M.D., CHICAGO, ILL.

To evaluate more completely the clinical significance of chronic gastritis in patients with gastrointestinal symptoms, a comparative analysis was made of the symptomatology and of the gastric secretory response to histamine among four groups of cases: (1) fifty patients with atrophy of the gastric mucosa, (2) fifty with superficial gastritis, (3) fifty with hypertrophic gastritis, and (4) 100 individuals in whom the gastric mucosa appeared normal gastroscopically. The absence of other organic disease was established by normal physical examinations, blood counts, urinalyses, normal proctoscopies, and by normal x-rays of the gastrointestinal tract.

The symptoms, with few exceptions, did not vary significantly among the four groups. The incidence of epigastric pain, the most frequent complaint, ranged from 44 per cent among patients with atrophy to 72 per cent among the normal subjects. The type, location, and pattern of pain were not distinctive for any of the four groups. There were, likewise, no significant differences in the incidence of nausea, vomiting, anorexia, constipation, weight loss, weakness, bad taste, or sore tongue. Diarrhea was described by 22 per cent of patients with atrophy as compared with 4 to 12 per cent for the other groups. The incidence of hematemesis in hypertrophic gastritis was 6 per cent, superficial gastritis, 2 per cent, and none in the remaining groups. A history of melena was encountered in 4 to 6 per cent of patients with gastritis and in one of the normal patients. Numbness or tingling of the extremities was reported by 6 per cent of patients with atrophy of the gastric mucosa and in 0 to 2 per cent of the remaining subjects. The duration of symptoms was essentially the same in all four groups.

Histamine achlorhydria was present in 51 per cent of forty-seven patients with atrophic gastritis, 27 per cent of forty-one with superficial gastritis, 9 per cent of forty-five with hypertrophic gastritis, and 3 per cent of ninety-five normal persons. A quantitative analysis of histamine tests performed in 165 patients indicated, as might be expected, that the smallest amounts of acid were secreted by patients with atrophy of the gastric mucosa, with superficial gastritis next. The output of acid in patients with hypertrophic gastritis, contrary to previous assumptions, did not differ significantly from that of normals.

The present data indicate that chronic gastritis is not an important cause of gastrointestinal symptoms.

97. BLEEDING PEPTIC ULCER; A REPORT OF ONE HUNDRED SIXTY CASES

GEORGE C. TURNBULL, M.D., AND EDWIN S. BRADEN, JR., M.D. (BY INVITATION) EVANSTON, ILL.

In 1938 a report was made on the eighty patients with bleeding peptic ulcer admitted to Evanston Hospital in the decade 1928 to 1937. The present report is an analysis of the 160 patients with bleeding peptic ulcer admitted in the succeeding decade, 1938 to 1947. It is made to evaluate further trends in management and results thereof.

The results of the present analysis indicate that the starvation (or strict Sippy) regimen is falling into disuse in a general hospital practice. The early use of a relatively liberal and balanced diet has found favor with most attending physicians. The results of this policy are shown by the further reduced time of bleeding, reduced amount of bleeding, lessened emergency surgery, shortened hospital stay, and lowered mortality.

In the present series there is no instance of surgery to control bleeding, and only three had elective surgery, which was done after the hemorrhage and anemia were under control.

It is of interest to note that ascorbic acid blood levels were determined in fifty-seven cases of varying clinical background. A few had levels within normal range, but the majority who had distinctly lowered levels of ascorbic acid in the blood showed the most prolonged and severe hemorrhage.

The increased availability of blood in recent years has led to the treatment of some of these patients with repeated transfusions (up to 6,000 c.c.). In this group there is no conclusive evidence of increased bleeding. On the other hand, there is no conclusive evidence that repeated transfusions are of value in shortening or reducing severity of hemorrhage, although they are the outsanding means of therapy for shock.

98. PYLORIC BALANCE IN ILEUS; CONCEPT AND APPLICATION

WILLARD BARTLETT, JR., M.D., ST. LOUIS, MO.

Ileus is a common clinical picture occurring as a complication of a variety of disorders, and the nature of the primary etiologic agent may become only a matter of speculation unless the patient is seen early in the episode. Mechanical small intestinal obstruction may supervene in peritonitis, or vice-versa; the factor of depletion of circulating protein by shifts into the peritoneal cavity and the edematous bowel wall occurs in each condition and further impairs intestinal tonus.

In the evaluation of the patient who is seen after several days of illness or in following the development of postoperative complications, any additional method of obtaining precise information is valuable. The application of continuous suction to the indwelling nasal catheter is of therapeutic merit, but if such decompression is used blindly and without consideration and measurement of the components involved, valuable information is not obtained, time is lost and actual harm (dehydration and demineralization of the patient) may be done. When properly employed, it affords a unique method of evaluating the degree of impairment of gastrointestinal function and yields data of great diagnostic and prognostic significance.

Quantitative studies begun by the author in 1930 led to the formulation of the term pyloric balance to express the volume and direction of flow of gastrointestinal secretions through the pylorus per day. In ileus, prognostic value lies in the fact that the quantity of the negative *balance* (lost to the body economy) varies characteristically with the nature of the crippling agent. Volumes greater than 1,500 c.c. are seen in mechanical small intestinal obstruction, rarely exceed 1 liter in peritonitis, and are less than 500 c.c. in the adynamic ileus that follows mere handling of the bowel at operation, particularly in patients with lowered circulating protein. With this factual knowledge, the crippling mechanism may be identified more certainly and appropriate treatment selected.

If a mechanical factor is clearly present, direct attack on the obstructing agent (band or volvulus) should be made if the general condition of the pa-

tient is good, particularly if the obstruction is jejunal. Enterostomy without exploration remains a most useful method of decompression of obstructed ileal loops, especially in the depleted patient or in those who are intolerant of prolonged nasal intubation. Distal emptying will occur after a few days as spasm and edema of the bowel wall at the site of obstruction subside and as kinking, plastic exudate is absorbed. Nasal catheter suction remains the treatment of choice for ileus in which no extrinsic mechanical factor is present, watch being kept for evidence of an unresolved abscess or infected hematoma which may require drainage.

99. DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS OF ACIDITY VARIATIONS IN THE STIMULATED AND NONSTIMULATED STOMACH

ROBERT B. SCHLESINGER, M.D. (BY INVITATION), LEO L. HARDT, M.D. (BY INVITATION), AND FREDERICK STEIGMANN, M.D., CHICAGO, ILL.

Alvarez and others have pointed out that the determination of the acidity in one gastric aspiration does not give reliable information concerning the gastric acidity status. Similarly, variations in the gastric acidity have been noted during fractional gastric analyses. In the present study we were impressed by marked variations in the acidity in the stimulated and unstimulated stomach not only from day to day but also during each fifteen-minute period and during the time of the experiment, and by their diagnostic and therapeutic implications.

Thirty-five patients with ulcer and four normal controls were tested for free and total acidity at fifteen-minute intervals for one- to two-hour periods daily for five to ten days under standard conditions. Gastric stimulation was produced by 0.1 c.c. of histamine diphosphate 1:1,000 solution per 10 kilograms of body weight.

Gastric acidity in nonstimulated ulcer patients varied from 25 to 143 units on successive days during the same period of time (8 to 10 A.M.). In the controls it varied from 0 to 19 units. Following histamine injections the acidity response varied from 6 to 96 units in the patients with ulcer and from 26 to 57 units in the controls. In one patient with gastric ulcer tested at almost weekly intervals, the acidity ranged from 0 to 29 units before and 11 to 62 units following histamine stimulation during a period of three months.

Gastric acidity varies at any given time over a period of days or weeks whether stimulated by histamine or not. These well-known variations may be caused by one of many influences acting upon an individual throughout the day, and should be taken into consideration when the therapeutic effect of any substance is evaluated, inasmuch as otherwise erroneous conclusions can be made by either condemning or approving a certain substance, depending upon the gastric secretory activity on that particular day.

Only prolonged observations on a large number of patients can give a clearer picture concerning the effect of a substance on gastric secretion. Although the daily variations are marked (as seen from the tables), the average obtained from a large sample (over 200 analyses) results in an almost flat curve.

In evaluating substances for ulcer therapy by testing them for their antacid effect, the periodic variations in gastric acidity occurring normally must be carefully considered.

100. GASTRIC ACIDITY RESPONSE DURING THE INTRAVENOUS ADMINISTRATION OF PROTEIN HYDROLYSATES

FREDERICK STEIGMANN, M.D., MITCHELL ZWEIG, M.D. (BY INVITATION), AND KARL A. MEYER, M.D. (BY INVITATION), CHICAGO, ILL.

The gastric secretagogue effects of protein and products of protein digestion when given orally are well known, but the effects of intravenously administered protein hydrolysates on gastric acid secretion have been studied by few workers. During an investigation of the clinical uses of protein hydrolysates, such observations on the gastric secretory response were made in a moderately large number of patients who received various types of protein hydrolysates with water or glucose as diluents.

After a control period of gastric secretion obtained during a saline infusion for forty-five to sixty minutes, one of the protein hydrolysates was infused at about 72 drops per minute, and the gastric aspirations continued for another hour at fifteen-minute intervals. Six types of protein hydrolysates (varying according to their preparation and diluent) were studied. Each proteolysate was tested a minimum of eleven and a maximum of twenty-four times, 162 tests being performed altogether.

All solutions stimulated gastric acidity to varying degrees and in varying percentages of the patients tested. Complete vagotomy (five patients) and thoracic sympathectomy and splanchnicectomy (three patients) did not change the foregoing observations. Plasma infusion caused no rise, while 10 per cent dextrose solutions caused a decrease in the gastric acidity.

The increase in gastric acidity during the intravenous infusion of protein hydrolysates may be due to various factors which require further investigation. The same proteolysates in a dextrose solution caused a smaller rise in gastric acidity and in a smaller number of patients. The pH of the solution seemed to be a factor also. Thus, the hydrolysates with a pH of 4 gave a higher acidity response than those with a pH of 6.5 to 7.0 (81 to 31 per cent, respectively, in forty-three tests). While the histamine content of these mixtures could be considered the cause of the gastric acid response following their intravenous administration, the results did not bear this out clearly. The hypoglycemia suggested by some observers as a cause for increased gastric secretion was not encountered in our patients.

These observations suggest that various known and some unknown factors may cause increased gastric acidity during protein hydrolysate infusions. In the clinical use of protein hydrolysates, therefore, one must attempt to eliminate at least the known various factors which possibly increase the gastric acidity, because such an increase may be harmful in some patients.

101. EXPERIENCES WITH NEEDLE BIOPSY OF THE LIVER IN HEPATIC CIRRHOSIS

R. C. COGSWELL, M.D. (BY INVITATION), F. CLEVELAND, M.D. (BY INVITATION), E. A. GALL, M.D. (BY INVITATION), F. WEISBROD, M.D. (BY INVITATION), AND L. SCHIFF, M.D., CINCINNATI, OHIO

Needle biopsy of the liver has revealed the presence of hepatic cirrhosis in sixty-six patients seen at the Cincinnati General Hospital during the past four and one-half years. The cirrhosis was classified as: nutritional (alcoholic) in thirty-seven, postnecrotic in seventeen, biliary in four, and was unclassified in eight. In the course of the clinical study, the following laboratory tests were

performed in most of the cases: cephalin-cholesterol flocculation, thymol turbidity, bromsulfalein excretion (5 mg. per kilogram of dye, retention in forty-five minutes), and serum bilirubin concentration. In over one-third of the cases total serum protein and albumin/globulin ratios were determined. The clinical diagnosis of cirrhosis was confirmed by the biopsy in fifty-five patients. In the remaining eleven, cirrhosis was first recognized as the result of the liver biopsy.

An attempt was made to correlate the histopathologic changes with the results of the various tests. The morphologic alterations were classified according to the degree of fatty vacuolization, cellular degeneration and necrosis, bile stasis, inflammatory reaction, and fibrosis. In the group of thirty-seven patients with nutritional (alcoholic) cirrhosis such a correlation study has been accomplished. Jaundice or hyperbilirubinemia was present in twenty of these patients; the thymol turbidity was increased in fifteen and normal in twenty; the cephalin-cholesterol flocculation was positive in thirty and negative in seven; bromsulfalein retention was present in twenty-nine and absent in five. Except for more marked fibrosis in the patients with either positive cephalin flocculation tests or abnormal bromsulfalein retention, there was no direct correlation between the results of the tests and the character of the histopathologic changes noted.

102. THE BLOOD AND BONE MARROW IN PATIENTS WITH CIRRHOSIS OF THE LIVER

LAWRENCE BERMAN, M.D. (BY INVITATION), ARNOLD R. AXELROD, M.D. (BY INVITATION), SAMUEL D. JACOBSON, M.D., THOMAS N. HORAN, M.D. (BY INVITATION), ELMORE C. VONDERHEIDE, M.D., AND ELWOOD A. SHARP, M.D.

DETROIT, MICH.

The peripheral blood and bone marrow findings in cirrhosis of the liver have been analyzed on the basis of a review of the literature and the authors' study of twenty-five patients with diagnoses verified by biopsy of the liver. The principal blood findings are macrocytic or normocytic anemia with normal or elevated mean corpuscular hemoglobin values, lymphopenia, and thrombocytopenia in the majority of the cases. Anemia may be independent of bleeding, and the severity of the anemia or macrocytosis does not appear to be related to the severity or duration of the liver lesion, although this appears to be true of experimental cirrhosis in rats. Absolute lymphopenia, regardless of the total leucocyte count, is the most constant significant alteration of the leucocyte picture.

The consistent change in the bone marrow is extension of the marrow organ so that active hematopoiesis is found in the shafts of the long bones. Regardless of the presence or absence of bleeding or anemia, the marrow of the sternum is of normal or increased cellularity, with normal or increased erythrocytogenesis and megakaryocytogenesis in most cases.

Even in patients with advanced liver lesions hypocellularity of the marrow is an unusual finding, in spite of peripheral anemia which is often characterized by a lack of signs of accelerated regeneration of red cells.

Macronormoblastic erythropoiesis is seen in patients with macrocytic anemia, but megaloblastic erythropoiesis does not result from hepatic cirrhosis.

The presence of peripheral cytopenias (anemia and thrombocytopenia) in spite of normal or increased formation of erythroblasts and megakaryocytes in the marrow is suggestive of hypersplenism. The well-known involvement of the spleen in patients with hepatic cirrhosis is additional evidence in favor of this view.

In patients with chronic hemorrhage, the blood and sternal marrow pictures are those of iron deficiency anemia, although other changes such as lymphopenia and thrombocytopenia tend to persist.

The changes described in the blood and bone marrow are not considered pathognomonic of hepatic cirrhosis, even though they appear to be characteristic of the disease. The combined blood and sternal marrow study is useful in establishing the diagnosis of cirrhosis of the liver in patients in whom other diseases have obscured its manifestations or in whom historical evidence was absent so that the clinical diagnosis was difficult to make.

103. THE ROUTINE USE OF THE SERUM FLOCCULATION REACTION WITH HAYEM'S SOLUTION

EMANUEL E. MANDEL, M.D., AND DELMO A. PARIS, M.D., CHICAGO, ILL.

(INTRODUCED BY HANS POPPER, M.D.)

A serum flocculation test with Hayem's* solution has been described by Gros as being a fine indicator of hepatic disease. The simplicity of his method appeared to justify an attempt at using it as a screening test. A modification of the original Gros test, necessitated by apparent shortcomings, was employed routinely in a series of 500 patients. The technique consists chiefly in mixing equal volumes of serum and Hayem's solution. Development of a precipitate within twenty-four hours is considered a positive result.

Seventy-two patients had a positive H (Hayem's) test. They may be classified as follows: acute hepatitis, five; cirrhosis of liver, ten; prolonged obstructive jaundice (two to six months), three; neuro- and cardiovascular syphilis, one; syphilitic chancre, two; active infectious or rheumatoid arthritis, eight; acute rheumatic fever, one; severe congestive failure, two; chronic lung infections (including pleuritis), fourteen; acute infectious diseases, fourteen; neoplastic diseases, eight; pyonephrosis, one; thyrotoxicosis, two; and scleroderma, one.

In all cases of this group and in many of the H-negative group, one or more of the following tests were carried out: sedimentation rate, cephalin flocculation, thymol turbidity, zinc sulfate turbidity, and Takata-Ara. The incidence of abnormal results of these tests was considerably higher in the H-positive than in the H-negative group. Likewise, hyperglobulinemia was far more frequent in H-positive than in H-negative sera. A significant increase in gamma globulin as determined by the Cohn-Wolfson method was found in all of the thirty-five H-positive sera in which this determination was performed.

In some instances a positive H-reaction was the only pathognomonic sign at the time of the patient's first examination, followed sooner or later by the discovery of the responsible disease (cirrhosis, metastatic neoplasm, pyonephrosis, pleuritis, active tuberculosis). No false positive reaction was encountered. All of these seventy-two patients were seriously ill even though the initial symptoms did not always betray this fact.

The H-negative group of 428 patients included a variety of conditions as they are encountered in the average hospital population. Among them were ten cases of acute hepatitis and three of (recent) obstructive jaundice, but none of cirrhosis of the liver.

The conclusion is warranted that the H test is capable of revealing diseases causing serum protein alterations. Hyperglobulinemia and particularly increases of gamma globulin appear to be chiefly responsible for a positive reaction.

*Mercury bichloride, 0.25; sodium sulfate, 2.50; sodium chloride, 1.00; distilled water, 100.00.

Consequently it may reveal not only a hepatic disorder but also systemic infections and malignant diseases. A negative test does not rule out any such conditions except perhaps cirrhosis of the liver. These findings and the simplicity of the test would favor its adoption as a routine clinical procedure.

104. THE INFLUENCE OF INGESTION OF VARIOUS LIPIDS UPON THE THYMOL TURBIDITY

HANS POPPER, M.D., FREDERICK STEIGMANN, M.D., HATTIE DYNIEWICZ, PH.C. (BY INVITATION), AND ALVIN DUBIN, M.S. (BY INVITATION), CHICAGO, ILL.

The thymol turbidity depends on the interplay of various factors among which the serum lipids appear the one most easily influenced experimentally. Hence the thymol turbidity was determined in 163 patients with various diseases before and three and six hours after the intake of various lipids. The turbidity rose, in general, after the ingestion of almost every type of lipid, the highest rise being encountered after administration of 50 Gm. of butter. The addition of 6 Gm. of choline to the butter usually enhanced the rise in the thymol turbidity. Choline alone elevated the thymol turbidity significantly less than butter with choline or butter alone. In five patients in whom the elevation of the thymol turbidity after the administration of butter and choline was compared with the rise of the serum phospholipids and total lipids, the slope of the thymol turbidity curve paralleled far more that of the serum phospholipids than that of the total lipids. The same was found in three patients in whom the response of the thymol turbidity, serum phospholipids, and total lipids was determined after the intake of either butter or choline alone. It was therefore concluded that the response of the thymol turbidity to the intake of choline or butter reflects primarily an elevation of the serum phospholipids. This is in keeping with the reported high concentration of phospholipids in the serum precipitate produced by the addition of thymol and by the known relation of choline to the serum phospholipids. The zinc sulfate turbidity—reflecting primarily gamma globulin (Kunkel) was determined in almost all instances simultaneously with the thymol turbidity; it represents an important supplement to the diagnostic use of the thymol turbidity and is to a much lesser degree influenced by lipid intake.

The alimentary rise of the thymol turbidity was on the average over 3 units in control subjects. It was depressed to a statistically significant degree (to about 1 unit) in infections, carcinoma, cirrhosis, and obstructive jaundice, and also in cardiac and wasting diseases. The smallest response, if any, was noted in gastrointestinal diseases. In acute infectious hepatitis the postprandial rise was almost normal. The response of thymol turbidity to the intake of butter and choline can thus be considered as a simple clinical test of intestinal absorption of lipids, most probably reflecting the phospholipids.

105. COMBINATION OF FLOCCULATION TESTS IN THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE

FREDERICK STEIGMANN, M.D., HANS POPPER, M.D., AND BERNARD H. SHULMAN, M.D. (BY INVITATION), CHICAGO, ILL.

In 455 patients, 262 with jaundice, the cephalin-cholesterol flocculation, thymol turbidity, and thymol flocculation were performed, and in almost all of them also the Takata-Ara, colloidal red, Gros (flocculation with Hayem's solution), and zinc sulfate turbidity (Kunkel) tests.

Most sensitive in the recognition of liver damage, in order of decreasing frequency, were the Gros test, the zinc sulfate turbidity, thymol turbidity, colloidal red test, cephalin-cholesterol flocculation, thymol flocculation, and Takata-Ara test.

In general, all flocculation tests were abnormal in a much higher percentage of patients with medical (patent extrahepatic biliary tract) than with surgical (obstructed extrahepatic biliary tract) jaundice. However, the number of false positive or negative tests with each individual test was too high to permit much reliance on the results of individual tests in the differential diagnosis. Purulent hepatitis produced by bacterial infection of the portal triads shows, though surgical in nature, the same results in the flocculation tests as the medical types of hepatitis. However, liver cell damage caused by biliary obstruction alone (biliary hepatitis) reveals, as a rule, negative or only slightly positive flocculation tests. If the possibility of a purulent hepatitis which can be recognized clinically by signs of septicemia is considered, the diagnostic value of the individual flocculation tests is somewhat increased. If the results of cephalin flocculation, thymol turbidity, Gros test, and zinc sulfate turbidity are dovetailed with each other by bringing them into a system which considers the degree of alteration of these tests, the percentage of those wrongly diagnosed as medical (false positives) was reduced to 3.5 per cent and of those wrongly diagnosed as surgical (false negatives), to 13 per cent of all jaundice cases.

In the differentiation of cirrhosis from infectious or toxic hepatitis, zinc sulfate turbidity and Gros tests are of particular value; they are highly pathologic in cirrhosis even with only slightly increased cephalin flocculation and thymol turbidity and moderately pathologic in acute hepatitis even if cephalin flocculation and especially thymol turbidity are greatly elevated. The Takata-Ara test is positive in cases with highly elevated zinc sulfate turbidity.

A quantitative correlation of the results of several flocculation tests may be useful for the differentiation between medical and surgical jaundice on the one hand and acute and chronic hepatitis (cirrhosis) on the other.

106. INFLUENCE OF ENDOGENOUS AND NUTRITIONAL FACTORS UPON THE PLASMA VITAMIN A ALCOHOL

HANS POPPER, M.D., FREDERICK STEIGMANN, M.D., HATTIE DYNIEWICZ, PH.C. (BY INVITATION), AND ALVIN DUBIN, M.S. (BY INVITATION), CHICAGO, ILL.

The total plasma vitamin A level reflects only vaguely temporary changes in vitamin A nutrition (it is reduced only in prolonged deficiency), and is significantly influenced by endogenous factors, for example, liver diseases or infections. Vitamin A in plasma and tissues occurs in either the free (alcohol) or the esterified form. Four-fifths of the total plasma vitamin A is normally in free form, whereas the liver contains primarily esterified vitamin A.

The question arises as to the influence of nutritional as well as endogenous factors upon the fractions of the total plasma vitamin A. In 276 control subjects and patients suffering from various diseases, the vitamin A esters only rarely were decreased below the normal (around 10 μg per 100 c.c.) but were significantly increased in nephrosis and erratically increased in various illnesses, especially liver diseases. The alcohol fraction, however, was significantly reduced (from about 40 to almost 15 μg per cent per 100 c.c.) in carcinoma, malnutrition, wasting diseases, infections (such as pneumonia) and liver diseases, especially cirrhosis, in which the lowest levels (for example 2 μg per 100 c.c.) were observed. Under pathologic circumstances the esters may represent even under fasting conditions more than 90 per cent of the total plasma vitamin

A. Endogenous hypovitaminemia A, therefore, is caused almost entirely by a reduction of the alcohol fraction, the esters often even being elevated. This effect can be explained by impaired release of vitamin A alcohol from the damaged liver due to inactivation of vitamin A esterase which normally converts the liver vitamin A ester to alcohol. Presence of esterase could be demonstrated in human liver and serum.

Intake of 75,000 units of vitamin A in aqueous or oily menstruum raises the plasma vitamin A esters for several hours. They may then represent in controls 80 per cent of the total vitamin A since the alcohol level does not rise during this period (twelve cases). Prolonged effect on vitamin A nutrition, however, was reflected in the plasma vitamin A alcohol. In eighteen persons who were given alternately for one to two weeks a diet containing less than 100 units of vitamin A daily or a normal diet with daily supplements of 10,000 units vitamin A, the plasma vitamin A alcohol level was, as a rule, significantly higher during the supplemented period than during the vitamin A poor period. A few exceptions found under pathologic conditions require further explanation. The vitamin A esters did not reflect the vitamin A intake, often being elevated during the vitamin A poor period.

It is concluded that both endogenous and general nutritional factors influence primarily the vitamin A alcohol level and that the latter, being independent of postprandial effects, represents a more sensitive index of vitamin A nutrition than the total vitamin A level.

107. STUDIES OF THE INFLUENCE OF OXYQUINOLINE DRUGS ON GROWTH OF ENDAMOEBA HISTOLYTICA AS MEASURED IN BLOOD IODINE LEVELS OF MAN

ALVA A. KNIGHT, M.D., AND JEANNE MILLER, M.A., CHICAGO, ILL.

(INTRODUCED BY ROBERT W. KEETON, M.D.)

In the study of iodine absorption in the oxyquinoline drugs in man, we found blood levels of iodine to be approximately 100 gammas in Anayodin and chiniofon, 400 gammas in Vioform, and 800 gammas in Diodoquin.

We then began the study of growth curves of *Endamoeba histolytica* in mixed bacterial flora in media consisting of equal parts of human serum and egg yolk infusion. Counts of both the amoeba and the bacteria in the inoculum were made, and these counts were repeated in the inoculated media at twelve, twenty-four, forty-eight, seventy-two, and ninety-six hours. The peak growth of *E. histolytica* occurred at forty-eight to seventy-two hours, while the bacteria continued to grow profusely for a longer period without diminution.

The patient was then given one of the oxyquinoline drugs for seven to ten days and the blood again drawn and its iodine content in gammas determined. Culture media of equal parts of serum and egg yolk infusion was again prepared and the inoculations made as before. The counts of *E. histolytica* and bacteria were made at the same time intervals as before. A total of over forty patients was studied.

Inoculated media containing 100 gammas of iodine showed a slow growth of *E. histolytica* continuing to seventy-two and ninety-six hours, but not the continuing rise to forty-eight or seventy-two hours previously shown. Growths became less active as the iodine level rose, and between 250 and 300 gammas no growth beyond forty-eight hours was shown. Levels of 400 to 600 gammas showed a progressively shorter growth period—rarely was any growth present at twenty-four hours.

The bacterial growth was uninfluenced by the varying iodine levels.

Studies in fecal concentrations of iodine have been made on a few patients. Other iodides are being studied in like manner and are to be reported on at a later date.

108. THE CLINICAL EVALUATION OF DUODENAL SUBSTANCE IN CHRONIC ULCERATIVE COLITIS

MICHAEL H. STREICHER, M.D., CHICAGO, ILL.

Duodenal substance was administered to thirty-five patients who had chronic ulcerative colitis. The dosage used varied from 12 to 48 tablets daily, each ½ Gm., administered uninterruptedly for one year. No other specific medication was used. The patients were on a controlled diet.

Comparative clinical evaluations were made as to the frequency of exacerbations in the disease before and after intake of duodenal substance, frequency of stools, presence of blood in the stools, gain or loss in weight, exaggeration or alleviation in abdominal cramping, and the general well-being of the patients under treatment.

The results obtained in 85 per cent of the patients were very favorable:

1. Exacerbation of the disease was noted in three instances.
2. The majority of the patients gained weight.
3. The majority of the patients felt better and ate better while receiving duodenal substance.
4. Duodenal substance may be considered a very valuable aid in the therapy of chronic ulcerative colitis.

109. THE CYTOLOGIC DIAGNOSIS OF CANCER OF THE STOMACH; PRELIMINARY REPORT

JEROME M. SWARTS, M.D. (BY INVITATION), ARTHUR BERNSTEIN, M.D. (BY INVITATION), ALEX B. RAGINS, M.D. (BY INVITATION), AND JACOB MEYER, M.D., CHICAGO, ILL.

The need for additional diagnostic methods for the purpose of detecting gastric neoplasms is well known. While the tested techniques of x-ray and gastroscopy are adequate in most cases, the results of these examinations not infrequently are equivocal or negative in the presence of a malignant tumor of the stomach.

The recent application of the cytologic technique in the diagnosis of cancer of the uterus has given impetus to wider application of the method in the diagnosis of neoplasms of the respiratory and genitourinary tracts. This method has been further utilized to a limited degree in the detection of carcinoma of the stomach.

In undertaking this study it was our purpose to evaluate its effectiveness both as an individual test and as a test complementary to roentgenography and gastroscopy in a large general hospital.

The method employed has been that of fasting gastric lavage with physiologic salt solution. The sediment obtained is smeared on glass slides, the smears being relatively thick. The stain which we have found most reliable is EA 36 (Papanicolaou). In addition we have also used the method of formalin fixation and paraffin section, staining the latter with stain EA 36 and with the standard hematoxylin and eosin stains.

All of the patients studied had clinical histories suggesting the possibility of gastric neoplasms. In the beginning we employed thin smears in forty-nine

instances, but because of the paucity of cells, this technique was abandoned. This report is based upon the study of 175 cases employing the thick smear technique. The paraffin section method has been used concomitantly in approximately 150 of these cases. Diagnoses in these cases included (1) carcinoma of the stomach, (2) gastric ulcer, (3) duodenal ulcer, (4) benign gastric neoplasms, and (5) gastritis and a variety of extragastric diseases.

Of the 175 cases, about thirty-five cases of gastric carcinoma are included. We have been able to make definitely positive diagnosis in approximately 35 per cent of these cases. Those cases of carcinoma of the stomach in which we were unable to make a positive cytologic diagnosis were cases in which the smears, because of the presence of debris and poorly preserved cells, allowed no interpretation. The two most important factors which prevent our making a positive diagnosis are (1) gastric retention and (2) the presence of large, necrotic tumors. The other factor is the infiltrating type of tumor without mucosal involvement. The proportion of false positive reports should not exceed 10 per cent, and we feel that this figure will be reduced even further. The results of the paraffin section studies parallel those of the smear study; the hematoxylin and eosin stains seem to be preferable when the former technique is used. In several instances we failed to find tumor cells preoperatively, and washings of the resected specimens also failed to reveal malignant cells.

This is a preliminary report. The series is as yet too small and the method too new to give statistically valid results. In the individual case, however, when other methods of examination give equivocal or negative results, a positive cytologic diagnosis may solve the problem.

110. COMPARATIVE NITROGEN BALANCE STUDIES OF ORAL PROTEIN DERIVATIVES AND NATURAL PROTEIN IN PROTEIN-DEFICIENT PATIENTS

DONALD D. KOZOLL, M.D., AND GORDON MACNEAL, M.D. (BY INVITATION)
CHICAGO, ILL.

Abundant evidence exists proving that oral protein digests are both practicable and biologically efficacious. A question which logically arises is: if patients are capable of ingesting a processed protein, what advantages does such a preparation offer over natural protein? A metabolic study in which synthetic and natural proteins are evaluated in the same protein-depleted patient has not, to our knowledge, been presented.

The opportunity to conduct such a study on three severly hypoproteinemic and hypoalbuminemic subjects presented itself in the course of a metabolic project designed to determine the relative biologic indices of a preparation of gluten with and without 1 per cent lysine. After having been on a regimen of protein therapy calculated to produce bare nitrogen equilibrium for periods of from thirty-four to fifty-five days, the patients showed no signs of clinical improvement or of regeneration of serum proteins or albumins. The patients were then given gluten with 1 per cent lysine* to the point of tolerance for periods of from eight to ten days, then equivalent amounts of natural proteins such as meat, eggs, and milk, and finally they were allowed diets combining maximal quantities of both. Daily nitrogen analyses of urine, stool, and ingested protein were carried out. Body weight, total serum protein, and serum albumin determinations were made at the beginning and conclusion of each period.

*Provided by Interchemical Corporation.

Results.—The maximal quantities of gluten with 1 per cent lysine ingestable averaged 22.8, 26., and 28.6 Gm. nitrogen per day for patients weighing 44.3, 52.0, and 55 kilograms respectively. This produced positive nitrogen balances of 9.3, 9.4, and 10.9 Gm. nitrogen per day respectively.

The maximal quantities of natural protein ingested ran 24.8, 26.5, and 28.6 Gm. nitrogen per day in the same order as previously given. The positive nitrogen balances were 7.5, 11.8, and 9.7 Gm. nitrogen per day in the order described.

The patients were able to ingest mixtures of the two sources of protein in the average ratio of two parts of the protein derivative to one part natural protein. Accordingly, 51.0, 49.7, and 55.8 Gm. nitrogen per day, respectively, were ingested (on the basis of body weight this was 6 Gm. or more protein per kilogram per day). This produced positive nitrogen balances of 18.8, 18.4, and 30.6 Gm. nitrogen per day respectively.

Total serum protein concentration *increases* for all three periods were 1.5, 3.3, and 2.2 Gm. per 100 c.c. respectively. *Increases* in serum albumin concentration were 1.1, 2.1, and 2.0 Gm. per 100 c.c. respectively.

Gains in body weight of 6.1, 7.0, and 6.3 kilograms, respectively, were noted at the end of the experiment.

Conclusions.—

(1) In two out of three protein-depleted patients, gluten with 1 per cent lysine produced a significantly better nitrogen balance than did similar quantities of natural protein. In a third patient the reverse was true.

(2) Combining a protein derivative with natural protein enabled the patients to ingest considerably more nitrogen than possible with either one alone.

(3) The ingestion of 6 or more grams of protein per kilogram per day was therapeutically feasible.

(4) Significant responses in total serum proteins, serum albumin, and body weight were seen.

111. EXPERIENCES WITH A MERCURIAL DIURETIC FOR SUBCUTANEOUS INJECTION

JOSEPH F. BORG, M.D., AND DAVID M. CRAIG, M.D. (BY INVITATION)
ST. PAUL, MINN.

A new mercurial diuretic, prepared for subcutaneous administration, has been tried clinically in office and hospital practice. Thiomerin is an organomercury compound combined with mercapto acetate, forming a mercaptide. It is a readily soluble white powder, which is diluted with water to contain 40 mg. of mercury per cubic centimeter. Preliminary reports show it to have a low incidence of local or systemic toxicity in animals.

The present report includes observations on thirty patients who have received from 1 to 50 subcutaneous injections of Thiomerin. The patients had all been under our observation, and most of them required frequent intravenous injections of one of the mercurial diuretics for control of congestive failure. Patients were maintained on ammonium chloride in dosage of 4 to 6 Gm. daily preceding and during the course of the present observation. Digitalis was continued if the patient was previously digitalized. Changes in the morning weight and also clinical observations of edema and pulmonary congestion were used as criteria of diuretic effect. The dosage was usually 1 c.c., but ranged from 0.25 to 2 c.c. Following injection, diuresis began in two to six hours and lasted from twelve to forty-eight hours. The weight loss was proportional to the

amount of edema and was usually two to five pounds, with extremes of zero to fifteen pounds. Three patients, followed for three months, have maintained a steady edema-free weight by substitution of Thiomerin for intravenous mercurials.

The injections have been surprisingly free from local reaction. One patient developed a sore nodule, still present after one month; another complained of local tenderness, lasting one week. About one-third of the patients experienced systemic symptoms, ranging from slight muscle cramps to severe prostration, cramps, and diarrhea, lasting from twelve to seventy-two hours. The more severe reactions were associated with greater urine output, although there was considerable individual variation. Usually subsequent injections, even with comparable diuresis, were attended by lesser or absent reactions. In four patients the use of Thiomerin was discontinued because of the severity of the symptoms. We have found Thiomerin a satisfactory diuretic for subcutaneous administration in the majority of patients. There is a low incidence of local reactions. Systemic reactions are more frequent than are seen after intravenous mercurials. When these occur, they are related to the degree of diuresis and can usually be prevented by the use of smaller doses.

112. RENAL EXCRETION OF SALICYLATE DURING TREATMENT OF ACUTE RHEUMATIC FEVER

WILLIAM S. HOFFMAN, M.D., AND CATHERINE NOBE, B.S. (BY INVITATION)
CHICAGO, ILL.

Clearance studies were carried out in twelve patients who were convalescing from acute rheumatic fever but were still taking large doses of aspirin (6 to 9 Gm. daily). Urine salicylate was partitioned into total salicylate, free salicylate, and salicylurate by the method of P. K. Smith and colleagues. Chloride and creatinine clearances were likewise determined. After these tests the subjects were placed on an alkalinizing regimen with doses of 2 to 3.3 Gm. of sodium bicarbonate every six hours for three days in addition to the regular aspirin medication. The clearance tests were then repeated. The urinary pH was noted in both sets of experiments. Plasma salicylate was found to be practically entirely unconjugated and was calculated as if it were all unbound, even though from 60 to 90 per cent has been found by other investigators to be bound to protein. To rule out the effect of alkali on the glomerular filtration rate, the apparent free salicylate clearances were expressed as ratios of salicylate to creatinine clearance ($SA/C \times 100$), as were chloride clearances ($Cl/C \times 100$).

During the initial tests, when the urine pH ranged from 4.98 to 5.99, $SA/C \times 100$ ranged from 0.84 to 2.43, while $Cl/C \times 100$ ranged from 0.45 to 3.16. In other words, the free salicylate ions which were filtered by the glomeruli were resorbed by the tubules almost as completely as chloride, if the urine was strongly acid. In the second set of experiments, when the urinary pH ranged from 6.22 to 7.98, $SA/C \times 100$ ranged from 4.6 to 22.8 or from two to eight times as high as when the urine was acid. The true clearance of unbound plasma salicylate would have been three to five times higher than these values. In other words, in the presence of excess sodium ions, free salicylate was cleared almost as well as mannitol. This phenomenon would account for the lowering of plasma salicylate levels when alkali is given.

The ratio of free salicylate to total salicylate in the urine was found to be proportional to the pH of the urine, being as low as 0.10 when the urinary pH

was about 5.0 and as high as 0.66 when the pH was 7.75. Thus alkalinization did not affect the excretion of conjugated salicylates, which were apparently already maximally high. In fact, with the absence of detectable amounts of conjugated salicylates in the plasma, it appeared that conjugated salicylates were either formed in the renal tubules or secreted by them like diodrast or hippurates.

Chloride-creatinine clearance ratios were not found consistently increased after alkalinization. Since the excretion of chloride is affected by many factors which could not be controlled in these experiments, these results are not suprising. Yet the dependence of both chloride and salicylate excretion on the amount of fixed base available for excretion would make it appear that both compete with each other for the available base and that under the conditions of acid urines, both are for the most part retained. This might explain the high plasma chloride and relatively low sodium concentrations found during salicylate therapy.

113. THE RECORDING OF OXYGEN SATURATION OF HEMOGLOBIN BY A WHOLE-BLOOD OXIMETER AND ITS APPLICATION TO CLINICAL INVESTIGATION

DALE GROOM, M.D., EARL H. WOOD, M.D., AND HOWARD M. ODEL, M.D.
ROCHESTER, MINN.

(INTRODUCED BY H. B. BURCHELL, M.D.)

A photoelectric oximeter has been devised, by means of which the degree of oxygen saturation of whole blood can be measured instantaneously and continuously, independent of the total content of hemoglobin, with a degree of accuracy sufficient to warrant its use in place of the Van Slyke gasometric analysis procedure in a variety of clinical and research applications.

The method is based primarily on two physical principles: (1) that of measurement of concentration of the chief blood pigments, oxyhemoglobin and reduced hemoglobin, in terms of their transmission of light according to Beer's law and (2) the differential light-absorption characteristics of these pigments in two spectral regions.

Whole blood, either in the form of a small sample or in continuous flow, is contained in a polythene tube which has an internal volume of 0.5 c.c. and is transilluminated by a constant-intensity light source. The emergent light is recorded in two selenium barrier-layer photoelectric cell circuits, one filtered to pass the red light wave lengths (650 to 750 millimicrons), which are transmitted almost exclusively by oxyhemoglobin, the other filtered to record in the near infrared (750 to 1,000 millimicrons), where both oxyhemoglobin and reduced hemoglobin transmit with approximately the same facility. Since the red cell current represents blood concentration of oxyhemoglobin and the infrared cell current that of total hemoglobin, a ratio of red to infrared cell currents constitutes a measure of the relative concentration of the two pigments and therefore of the relative degree of oxygen saturation of the hemoglobin. By empirical calibration of the apparatus against results obtained by the Van Slyke method, absolute values, in terms of percentage of oxygen saturation, can be read directly from a nomogram or from a single calibrated galvanometer scale. The method also lends itself to photographic recording of saturation values, synchronously and continuously with recordings of other physiologic variables.

To date this instrument has been utilized chiefly for studies of oxygen saturation of arterial blood and, in diagnostic cardiac catheterization where it allows immediate correlation of roentgenographic, intracardiac pressure and oxygen saturation observations.

Accuracy of this photoelectric method has been computed from the results of simultaneous photoelectric and Van Slyke determinations carried out on 131 blood samples ranging in hemoglobin concentration from 13.0 to 25.8 Gm. per 100 c.c. The standard deviation of the differences between values obtained by the two methods was 1.8 indicated saturation per cent; this implies a 95 per cent likelihood that any saturation determination made by the whole-blood oximeter will be within 3.6 per cent saturation of that obtainable by Van Slyke analysis.

114. THE DETERMINATION OF HEMOGLOBIN AS PYRIDINE HEMOCHROMOGEN

A. Hughes Bryan, M.D., T. Franklin Williams, M.A. (By Invitation), and Celia R. Webb, A.B. (By Invitation), Chapel Hill, N. C.

For use in nutrition surveys a hemoglobin method was sought which would be accurate, reproducible, and convenient to use in field studies, allowing the blood to be analyzed in a central laboratory some hours after collection in the field. Of the many published hemoglobin methods, we selected the pyridine hemochromogen method of Rimington, modified it, and adapted it to the Beckman spectrophotometer and the conditions imposed by field studies.

Method.—Ten cubic millimeters of blood measured in a calibrated Sahli-type pipette are discharged into 1 c.c. of distilled water in a tube calibrated to contain 5 c.c. The resulting hemolyzed blood is allowed to stand at room temperature twenty-four hours before analysis.

The pyridine hydrosulfite reagent is prepared fresh for each batch of analyses as follows: Five grams of sodium hydrosulfite powder is dissolved in approximately 80 c.c. of N/10 sodium hydroxide. The solution is filtered, 5 c.c. of pyridine are added, and the combined reagent diluted to 100 c.c. with N/10 sodium hydroxide.

To the hemolyzed blood are added 0.5 c.c. of pyridine hydrosulfite reagent and 1 c.c. of N/5 sodium hydroxide, and the reaction mixture is diluted to 5 c.c. with N/10 sodium hydroxide. The tube is shaken after each addition and foaming can be dispelled by the use of one drop of n-butyl alcohol. The density is determined in the Beckman spectrophotometer after ten to sixty minutes at 558 to 560 mμ. Pyridine hemochromogen has a very sharp absorption peak in the neighborhood of 558 to 562 mμ. For accurate work it is necessary to determine the point of maximum absorption for each set of determinations. The hemoglobin content of the blood is obtained from a calibration curve prepared by submitting to the foregoing procedure bloods whose hemoglobin content was determined by analysis for iron. A straight line relationship exists between hemoglobin content and density of pyridine hemochromogen.

We have investigated the influence of the following variables: (1) proportions of sodium hydrosulfite and pyridine in the reaction mixture, (2) effect of the order of adding the reagents, (3) change of absorption with time after adding the reagents, and (4) effect of the time of standing of the hemolyzed blood before addition of the reagents. The conditions described appear to give the most satisfactory and reproducible results. Preliminary work indicates that the error in repeated single determinations on the same blood is less than ±3 per cent.

115. THE CELLULAR COMPOSITION OF THE BONE MARROW IN NORMAL INFANTS AND CHILDREN

KURT GLASER, M.D. (BY INVITATION), HENRY G. PONCHER, M.D., AND LOUIS R. LIMARZI, M.D., CHICAGO, ILL.

Extensive reviews of the literature on the cellular composition of the bone marrow in children have shown the lack of information available on this subject and have expressed the need for further investigation.

This study covers 138 sternal marrow punctures on normal individuals from birth to 19 years of age. There was very little, if any, variation by age found in the cell counts of individuals ranging in age from 1 to 19 years. The findings of the cell counts of this age group were therefore used to establish normal average values for the cellular distribution of the bone marrow (myeloblasts, 1.23 per cent; leucoblasts, 1.44 per cent; promyelocytes, 1.8 per cent; myelocytes, 3.46 per cent*; metamyelocytes, 36.05 per cent; polymorphonuclear cells, 12.9 per cent; eosinophiles, 3.58 per cent; pronormoblasts, 0.47 per cent; basophilic normoblasts, 1.69 per cent; polychromatic normoblasts, 18.2 per cent; orthochromatic normoblasts, 2.72 per cent).

Cell counts on infants less than 1 year and particularly less than 1 month old showed wide and constant variations with age. The number of nucleated red cells during the first and second days of life approximated those established as normal averages (23.12 per cent). From then up to the fifteenth day there was a marked decrease, followed in turn by an increase to average values. Lower figures were again recorded during the third to fifth months. Among the individual cells of this series the polychromatic normoblasts showed variations identical to those of the total red cell group. Pronormoblasts and basophilic and orthochromatic normoblasts showed similar findings but the fluctuations in number became less marked and statistically less significant with the smaller number of cells. Over the entire span of nineteen years there seemed to be a tendency for the younger cells to decrease in number with progressing age and for the older cells to increase during the same period. This variation in percentage was minimal, yet it occurred with remarkable consistency.

The observations on the myeloid series likewise brought strong and constant changes during the first month of life, with less significant variations during later months and years. The values for the first and second days of life closely approached the average figures (60.59 per cent); the following days we saw a marked increase in number of myeloid cells, with a drop around the twentieth day, followed by a rise to average figures by the end of the first month. From then on there was little deviation from the average up to the end of the observation period (nineteen years). As to the individual cells in this group, the metamyelocytes, which form the largest part of the series, showed the same pattern as the total group. The smaller groups of the myeloid series showed findings similar to those of the total group, but again the variations were not as marked because these cells occurred in much smaller numbers. In considering the total period of observation there seemed to be again a tendency for the number of younger forms to decrease and for the number of older forms to increase.

The myeloid-erythroid ratio showed during the first and second days figures close to the established average (2.94), followed by a sharp rise up to the end of the second week (14.5), when the values again fell, reaching the average at the

*All cells showing more than kidney-shaped indentation of their nucleus were placed among the metamyelocytes, thus producing relatively low values for the myelocytes.

end of the first month. Thereafter the deviations of individual counts from the average bore no relation to the age of the child.

The lymphocytes varied in number from a low of 4 to 7 per cent on the first to sixth day to a high of 38 per cent around the twentieth day. During the first and second year there was a gradual decrease (corresponding to the decrease in the peripheral blood), reaching the average level (16.03 per cent) at around 4 to 5 years of age.

Basophilic granulocytes, plasma cells, megakaryocytes, monocytes, and reticulohistiocytes occurred in small numbers and no tendency of the numerical variations of these cells with regard to age could be noted in our counts.

Conclusion.—Normal cell values for the age period from birth to 19 years were established by means of 138 sternal marrow punctures. Constant and important variations in number of all cell groups were noted during the first month of life and of the erythroid series during the third to fifth months. No marked deviation from average values occurred during the remaining observation period. The scarce data available in the literature, usually limited by the small number of cases reported or inadequate age distribution or statistical evaluation, served nevertheless to confirm our findings. Further investigation as to the cellular composition of the bone marrow in older individuals, studied by the same method, seems indicated.

116. STERNAL MARROW STUDIES IN HODGKIN'S DISEASE

LOUIS R. LIMARZI, M.D., AND JEROME T. PAUL, M.D., CHICAGO, ILL.

It is generally agreed that Hodgkin's lymphogranulomatosis is not a disease of the lymph nodes exclusively but that the lesions may be widespread, resembling more closely the distribution of the reticuloendothelial system than that of any other system. Although the ultimate diagnosis may in many cases rest upon the histologic examination of the affected lymph node, 12 per cent or more of patients with Hodgkin's disease do not show any lymph node enlargement for a long period of time.

Interest in the value of sternal puncture in the diagnosis of Hodgkin's disease becomes apparent when one considers the high incidence of the marrow involvement in this disease. Although the vertebrae and pelvis seem to be primarily attacked, the sternum is involved in a high percentage of autopsy cases (63.7 per cent). It is of interest to note in view of the fact that most of the peripheral blood changes that have been described in Hodgkin's disease concern cellular elements of bone marrow origin that the first examination of the sternal marrow during life in this disease was made by Weiner and Kaznelson in 1926.

This report comprises the hematologic and clinical observations made on a group of thirty-five patients with Hodgkin's disease. Besides the normal controls, the blood and bone marrow were studied in patients with lymphosarcoma, reticulum-cell sarcoma, and mycosis fungoides. The bone marrow studies were done from both films and histologic preparations made from bone marrow particles of the marrow material obtained by sternal aspiration or biopsy material removed by means of small trephine.

From the results of our bone marrow studies during life and a review of the literature it is concluded that (1) the most constant findings in the bone marrow in Hodgkin's disease are a myeloid and megakaryocytic hyperplasia. The morphologic pattern of the bone marrow and especially that of the granulopoietic tissue is that seen in chronic toxic states. The panhyperplasia of the bone marrow observed in some cases simulates that of Banti's disease. (2) A pathologic type of megakaryocytopoiesis involving the "lymphoid" type of mega-

karyocytes is observed in some cases of Hodgkin's disease. These cells are not specific for this reason. These "lymphoid" megakaryocytes may produce atypical forms of platelets. (3) Reed-Sternberg cells were not seen in sternal aspiration material nor in the histologic sections of bone marrow particles from such material. Evidence is presented to show that the giant cells of Hodgkin's granuloma are not similar nor related to bone marrow megakaryocytes. (4) The finding of increased numbers of normal or atypical plasma cells, eosinophiles, and reticular cells is neither a constant nor a specific pattern of the bone marrow in Hodgkin's disease. Eosinophilia of the bone marrow cannot be correlated with the peripheral blood eosinophilia. (5) The accurate diagnosis of the disease is established only by the finding of the specific pleomorphic lesion of Hodgkin's granuloma. This is best observed in the histologic section of an involved lymph node. The finding of any one cell or group of cells in sternal aspirated bone marrow films is insufficient evidence for the diagnosis of Hodgkin's disease.

117. ANEMIA OF PREGNANCY TREATED WITH MOLYBDENUM-IRON COMPLEX

WILLIAM J. DIECKMANN, M.D. (BY INVITATION), HAROLD D. PRIDDLE, M.D. (BY INVITATION), ROSE TURNER, B.A. (BY INVITATION), AND BETTY TREPTOW, (BY INVITATION) CHICAGO, ILL.

Four and six tenths per cent of our pregnant patients have a hemoglobin concentration less than 10 grams per 100 ml., but, if the nonpregnant standard of 12 grams is used, 39 per cent are anemic. We believe that the lower limit of the hemoglobin concentration between the twelfth and thirty-sixth weeks is 10 grams and from then to term is 10.5 grams. Up to twelve weeks antepartum and by six weeks post partum the lower limit is the normal nonpregnant limit or 12 grams of hemoglobin. All patients are screened by a hematocrit determination every three months, at term, and again at six weeks and six months post partum. Any patients with hematocrits less than 37 for the nonpregnant or early pregnant or less than 30 between the twelfth and thirty-sixth weeks or less than 32 between thirty-six weeks and term have a hemoglobin determination, an erythrocyte count, and the various indices calculated.

Treatment with ferrous and ferric iron alone or with various vitamin combinations did not cause a significant increase in the rate of hemoglobin formation. A molybdenum-iron complex has resulted in a rapid increase in hemoglobin concentration. If this does not occur within three weeks, then more extensive studies should be made to determine the cause of the anemia.

118. OBSERVATIONS ON THE TREATMENT OF ACUTE LEUCEMIAS WITH ANALOGUES OF FOLIC ACID

W. JACOBSON, M.D., WILLIAM C. LEVIN, M.D., AND GORDON HOLT, M.D.
GALVESTON, TEXAS
(INTRODUCED BY RAYMOND GREGORY, M.D.)

Ten patients, belonging to the group with acute leucemia, have been treated with Aminopterin (4-amino-pteroylglutamic acid) or Methopterin (10-methyl-pteroylglutamic acid). Acute lymphocytic leucemia (leucosarcoma) was the diagnosis in seven instances, and acute monoblastic leucemia in three. Eight patients were children and two were adults.

In general the patients were treated with 2 mg. of the compound, administered intramuscularly, on alternate days. Some patients, receiving Methopterin, had the dosages increased to 2 mg. daily. Blood transfusions and antibiotics were administered when indicated.

Patients Treated With Aminopterin.—Three patients received Aminopterin only; a fourth patient received Aminopterin following an initial period on Methopterin. All four developed evidences of toxic reactions as manifested by stomatitis, macular rash, and marked depression of bone marrow activity. One of this group showed some clinical and hematologic improvement. One patient has died, and the remaining two have failed to show a definite response.

Patients Treated With Methopterin.—Methopterin was given to six patients and a seventh received Methopterin but later was treated with Aminopterin. Of these seven patients, two died before the drug had a chance to act. Of the remainder, none developed manifestations of toxic symptoms in contrast to the patients treated with Aminopterin. One patient has developed a very conspicuous clinical and hematologic remission with a reversal of the marrow to a nearly normal picture. Another patient improved while receiving Methopterin but her condition deteriorated when subsequently receiving Aminopterin. Three patients have so far shown no improvement but are still under treatment.

The impression has been gained that Methopterin is superior to Aminopterin in the treatment of acute leucemias, especially in view of its lower toxicity.

119. TREATMENT OF ACUTE LEUCEMIA WITH AMINOPTERIN (4-AMINO-PTEROYLGLUTAMIC ACID)

MILA PIERCE, M.D. (BY INVITATION), AND HOWARD ALT, M.D., CHICAGO, ILL.

Remissions characterized by clinical and hematologic improvement and a return of the marrow pattern to normal in acute leucemias treated with Aminopterin were reported by Farber and co-workers, in the spring of 1948. The duration of treatment in this preliminary report was of three months or less. This report deals with our experience in the treatment of eleven patients with acute leucemia since May 1, 1948.

The action of Aminopterin is not clearly understood. Farber observed certain pteroylglutamic conjugate, notably Teropterin and Diopterin, induced an accelerating effect on leucemic tissue. The search for an antagonist drug led to the synthesis of Aminopterin; this product caused a rapid fall in the total blood leucocytes, a reduction in the size of the hematopoietic organs, and aplasia of the marrow.

In our series there were nine children under 10 years of age, one adolescent, 13 years of age, and one adult, 66 years of age. All but one had acute untreated leucemia. The duration of the disease prior to treatment was less than one month in ten patients. The drug was administered intramuscularly. The daily dose was adjusted to the age and weight of the patient and varied from .25 to 1 mg. in the children and from 1 to 2 mg. in adults. Daily administration was continued until clinical and hematologic effects were obtained; thereafter treatment was given four to five times a week until toxic manifestations developed. Transfusions were used to support the anemia. Hematologic effects were followed by serial blood and bone marrow studies. The duration of treatment has extended more than four months in four cases, two months in three, one month in two, and less than one week in two. Of eleven patients treated, eight

are still living, three are dead. Of the eight living patients, five sustained remission; three did not. Of the three patients who died, one was treated in the terminal phase and one succumbed to a cerebral hemorrhage; one was the adult. In the five patients in whom complete remissions were obtained, the duration of the clinical and hematologic improvement lasted for more than six weeks in two instances, after which some degree of enlargement of the hematopoietic organ and/or return to the leucemic marrow pattern recurred. Both of these patients are still living. In three cases, the duration of the remission is less than a month. Of the three living patients who showed no remission, two were treated continuously four months and one for three weeks.

The remissions were initiated in each instance by a fall in the total number of blasts in the peripheral blood, and a rapid reduction in the size of the hematopoietic organs. The effects occurred within ten to twenty days after instituting treatment. Hyperpyrexia, abdominal tenderness, and severe hemorrhagic diatheses were often encountered. In all instances a severe aplasia of the marrow accompanied these signs. The total dose of Aminopterin administered before signs of marrow aplasia developed varied from 2.5 to 20 mg. The drug was discontinued when aplasia developed. Following the aplastic stage, the total leucocyte count rose gradually, followed by a rise in the reticulocytes, erythrocytes, and platelet levels. Granulopoiesis returned in the bone marrow within five to ten days. During the period of active marrow granulopoiesis, clinical improvement was dramatic and evidenced by a drop in the temperature curve and general appearance of good health. Treatment was resumed as soon as granulopoiesis was re-established. Stomatitis developed in seven patients under prolonged treatment. In the two under treatment for four months, who sustained a complete remission, relapse of the symptoms occurred after six weeks and the clinical course has been subacute. Relapse was initiated by increase in size of the peripheral lymph nodes and spleen, a rising white count, and recurrence of the leucemic marrow pattern. Under continued treatment reduction in the hematopoietic organs was obtained again. However, the bone marrow has remained leucemic. In spite of the marrow involvement, the red count, hemoglobin, and platelet values remained normal since the initial remission, a period now of more than three months. In the three patients under continuous therapy for four months, the clinical course has been subacute and organ involvement has been minimal, but no measurable improvement in the marrow pattern has been noted on repeated examinations.

Summary.—Of eleven patients with acute leucemia treated since May 1, 1948, remissions have been obtained in five instances. The remissions have been characterized by a severe marrow aplasia followed by a rapid regeneration. Remissions are not sustained. Whether the use of this product will significantly change the course of the disease remains to be proved.

120. TREATMENT OF CHRONIC LEUCEMIA WITH A FOLIC ACID ANTAGONIST

LAWRENCE BERMAN, M.D. (BY INVITATION), ARNOLD R. AXELROD, M.D. (BY INVITATION), ELMORE C. VONDERHEIDE, M.D., AND ELWOOD A. SHARP, M.D., DETROIT, MICH.

Previous reports on the use of pteroylglutamic acid antagonists in man have dealt primarily with the treatment of acute leucemia. This study is based on observations of the effect of the folic acid antagonist 4-amino-pteroylglutamic acid* in nine patients with chronic leucemia. One of the patients having my-

*N-[4-{[(2,4-diamino-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid.

elogenous leucemia received two series of treatments. The drug was taken orally, in daily doses ranging from 1.25 to 7.5 mg. over periods from six to twenty-five days. Results are shown in Tables I and II.

TABLE I. LEUCOCYTE COUNTS IN PATIENTS WITH MYELOGENOUS LEUCEMIA BEFORE AND AFTER TREATMENT

CASE	TOTAL W.B.C. PER C.MM.	STEM CELLS PER C.MM.	IMMATURE GRANULOCYTES PER C.MM.	MATURE GRANULOCYTES PER C.MM.	TOTAL DOSE IN MG.
1a	300,000	14,280	121,200	109,000	0
	15,000	0	856	7,450	117
1b	100,000	4,330	22,080	62,400	0
	68,000	302	15,410	37,200	49
2	150,000	7,810	72,000	49,500	0
	28,700	287	861	20,900	97
3	47,800	8,560	10,710	24,580	0
	18,850	296	1,059	14,320	97
4	263,000	27,920	98,600	100,800	0
	184,000	6,930	5,550	118,800	86
5	78,000	19,080	8,860	33,300	0
	5,750	39	78	3,921	67

TABLE II. LEUCOCYTE COUNTS IN PATIENT WITH LYMPHATIC LEUCEMIA BEFORE AND AFTER TREATMENT

CASE	TOTAL W.B.C. PER C.MM.	STEM CELLS PER C.MM.	GRANULOCYTES AND MONOCYTES	LYMPHOCYTES	TOTAL DOSE IN MG.
1	102,000	4,270	15,500	82,200	0
	73,800	5,740	260	67,800	132
2	76,400	0	12,500	63,900	0
	77,800	0	8,600	69,200	105
3	35,130	0	4,567	30,563	0
	50,000	0	10,500	39,500	52
4	231,000	26,240	6,644	198,100	0
	180,500	8,570	7,876	164,000	56

Stem cells in both myelogenous and lymphatic leucemia and immature granulocytes in myelogenous leucemia decreased in number, but platelet and erythrocyte counts were variable. Usually mature granulocytes decreased in number during treatment, but lymphocytes of mature type were not affected. Progressive falls in total leucocyte counts occurred for as long as ten days after treatment was stopped.

The most important morphologic effects included the appearance of small numbers of megaloblasts in material aspirated from the sternal marrow. The cells were indistinguishable from those characterizing erythropoiesis in addisonian pernicious anemia in relapse. They were seen after approximately 100 mg. of the medication had been ingested over periods from six to twenty-four days.

In two patients with lymphatic leucemia there were decreases in the sizes of lymph nodes. The hematologic effects of therapy were temporary and never completely adequate because treatment had to be stopped when toxic signs appeared.

The toxic effects, in the order of appearance, included pharyngitis, stomatitis, sore tongue, crampy abdominal pain sometimes associated with diarrhea, dermatitis, and alopecia. Usually pharyngitis first appeared within a few days before or after significant changes in the leucocyte counts were noted. The

total dosage of the drug which produced toxic effects varied from 30 to 75 mg., although one patient received 105 mg. without experiencing such signs. There was evidence that when small doses are used initially, a larger cumulative dose can be tolerated before toxic symptoms appear.

Autopsies on two patients who expired shortly after therapy was stopped revealed no changes which could be attributed to the medication.

Although 4-amino-pteroylglutamic acid had a definite hematologic effect, there was no subjective improvement in any of the patients. The use of the drug in chronic leucemia is limited by the early development of toxic phenomena. Further studies with related compounds are warranted.

121. EFFECT OF ANTIFOLIC ACID DERIVATIVES ON PATIENTS WITH FAR-ADVANCED CARCINOMATOSIS

SAMUEL G. TAYLOR, III, M.D., DANLEY SLAUGHTER, M.D. (BY INVITATION), FREDERICK W. PRESTON, M.D. (BY INVITATION), JOSEPH CRUMRINE, M.D. (BY INVITATION), AND GEORGE HASS, M.D., CHICAGO, ILL.

Observations were made on thirty-five patients with far-advanced carcinomatosis given pteroyltriglutamic acid (Teropterin) intramuscularly daily for from two weeks to twelve months. The dose varied from 20 mg. daily to 50 mg. b.i.d. The following diseases were treated: two epidermoid carcinomas, one carcinoma of the pancreas, three mixed tumors of the parotid, three adenocarcinomas of the cecum, two carcinomas of the colon, five carcinomas of the breast, three transitional cell carcinomas of the bladder, one thymic carcinoma, two bronchogenic carcinomas, one adenocarcinoma of the rectum with carcinoid of the ileum, two carcinomas of the ovary, one epithelioma of the penis, one hypernephroma, one neurofibrosarcoma, one lymphosarcoma, one fibrosarcoma of the orbit, two melanosarcomas, one Ewing's sarcoma, two unclassified malignant bone tumors.

No patients showed any evidence of toxic reactions to the drug even with very large doses. No change in the expected course of the disease was noted in thirty-four of the thirty-five patients given the drug. We were unable to demonstrate any beneficial effects attributable to administration of the drug in these patients.

One patient with pulmonary metatases from a carcinoma of the breast showed roentgenographic evidence of incomplete regression of the metastatic nodules after three months of daily injections of 40 mg. pteroyltriglutamic acid. Though the metastatic nodules persist, there has been no increase in their size five months following cessation of administration of the drug.

Autopsies were performed in twelve instances. No specific effect of the drug on tumors could be demonstrated microscopically.

Twelve patients with malignant disease were given 4-amino pteroylglutamic acid (Aminopterin). The following diseases were treated: one mycosis fungoides, nine carcinomas of the female breast, one adenocarcinoma of the ovary, one melanosarcoma.

Most patients developed ulcerative lesions in the buccal, vaginal, or rectal mucosa during therapy. Granulocytopenia occurred in two patients and a fatal pancytopenia developed in one. This patient also had multiple ulcerations of the gastrointestinal tract. Stomatitis and vaginitis usually preceded granulocytopenia. The appearance of toxic reactions frequently necessitated omission

of the drug. Stomatitis and leucopenia developed in one elderly patient after two daily injections of 1 mg. of 4-amino pteroylglutamic acid.

An arrest of rapidly progressive neurological changes and roentgenographic evidence of marked regression in the pulmonary lesions have been noted in one patient with metastatic carcinoma of the breast. Marked regression of regional metatases was noted in one other patient with metastatic mammary cancer. However, both patients experienced severe toxic reactions to the drug.

It is concluded that pteroyltriglutamic acid in the doses given are probably of no value in the treatment of the types of malignancy stated in the foregoing.

No conclusion can yet be drawn on the effect of 4-amino pteroylglutamic acid on malignancy. However, the dramatic response of one patient necessitates further careful observations despite the dangerous toxic side effects.

122. EFFECT OF VITAMIN B_{12} ON THE HEMATOPOIETIC AND NERVOUS SYSTEMS IN ADDISONIAN PERNICIOUS ANEMIA

B. E. Hall, M.D., and D. C. Campbell, M.D. (By Invitation),
Rochester, Minn.

The effect of vitamin B_{12} in six patients having classical Addisonian pernicious anemia has been studied. In four of the six patients the diagnosis of pernicious anemia had not been established previously. The remaining two patients had had short courses of treatment with parenteral liver extracts four and three years, respectively, prior to admission to the Clinic. All six patients were in severe relapse. Reticulocyte peaks were noted four to seven days after the institution of therapy and were followed by a rise in erythrocyte counts which reached normal levels in from four to six weeks. Serial aspirations of the bone marrow revealed complete conversion from megaloblastic to normoblastic regeneration in from forty-eight to ninety hours after administration of B_{12}. Lantern slides in color will be used to depict the serial changes in the marrow.

Excellent hematologic responses were obtained with doses of 25 μg of vitamin B_{12} administered at weekly intervals intramuscularly. Some evidence was obtained to indicate that intervals longer than one week between injections were not always accompanied by a maximal rise in erythrocytes. In one case an initial dose of 6 μg was not followed by a maximal reticulocyte response. An interesting observation was the development of hypochromasia in the erythrocytes of two of the six patients during the period of rise in erythrocyte levels. The significance of this observation is not clearly understood and needs further study.

Four of the six patients had glossitis prior to the institution of therapy. In all four the glossitis improved after treatment was begun and eventually disappeared. Four of the six patients also had definite evidence of subacute combined degeneration of the spinal cord. One patient showed no improvement symptomatically or objectively after receiving 100 μg of vitamin B_{12} over a period of sixty-two days. A second patient showed remarkable improvement after receiving 75 μg over a period of thirty-three days. The remaining two patients have not been observed long enough to permit conclusions to be drawn. It should be emphasized, however, that prolonged observation over a period of many months will be necessary before it will be possible to determine whether or not vitamin B_{12} therapy will protect permanently the central and peripheral nervous systems in pernicious anemia.

123. ESSENTIALITY OF BOTH THE ANTIPERNICIOUS ANEMIA FACTOR OF LIVER AND PTEROYLGLUTAMIC ACID FOR HEMATOPOIESIS IN SWINE

ROBERT W. HEINLE, M.D., ARNOLD D. WELCH, M.D., AND JACK A. PRITCHARD, M.D. (BY INVITATION), CLEVELAND, OHIO

Macrocytic anemia with megaloblastic hyperplasia of the marrow was induced in swine by use of a diet essentially free of extrinsic factor and pteroylglutamic acid (PGA) and containing a crude PGA antagonist and succinylsulfathiazole.

One pig thus rendered anemic responded incompletely to one dose of PGA-free liver extract (15 units) but two months later failed to respond to ten successive daily doses of 15 units each. Four 15 mg. doses of PGA then produced an excellent hematologic response.

Another pig failed to respond to five daily doses of 200 Gm. of crude casein, used as a source of extrinsic factor. In a second period, ten-minute daily doses (0.05 mg. each) of PGA caused only a small reticulocyte response. In a third period, however, five daily doses of 200 Gm. of crude casein produced a secondary reticulocytosis and a more complete hematologic response.

The findings indicate that a dual deficiency involving PGA and the antipernicious anemia (APA) factor was produced. In the first pig it is suggested that the first administration of liver extract produced a small response because PGA was not completely absent, while the second administration of liver extract was ineffective because the animal was now devoid of functional PGA. Whether a deficiency of APA factor had also been re-established cannot be stated, but failure to respond to liver extract indicates that the PGA-deficiency was, by itself, capable of preventing hematopoiesis. In the second pig it is suggested that extrinsic factor was ineffective until a catalytic amount of PGA was made available.

A third anemic animal responded twice to single doses of 1 mg. of PGA given at monthly intervals. However, a third dose, given after another month, failed to elicit a significant response. A single dose of 100 units of purified liver extract produced a good reticulocytosis and a partial hematologic remission. However, this restored the ability to respond to PGA, as indicated by a good reticulocyte response following a fourth injection of 1 mg.

The results in this animal indicate that deficiencies of PGA and of the APA factor were produced. When the deficiency of APA factor became sufficiently profound, PGA was unable to induce hematopoiesis. With removal of the deficiency of the APA factor, with a resultant partial remission, PGA again became hematopoietically active.

It may be concluded that under the conditions of these experiments neither the APA factor nor PGA can function hematopoietically in the absence of the other and that both substances are essential to the processes involved in the maturation of erythrocytes.

124. FURTHER STUDIES ON THE EFFECT OF URETHANE ON LEUCEMIA

PAUL L. BEDINGER, M.D., CHICAGO, ILL.

(INTRODUCED BY LOUIS R. LIMARZI, M.D.)

Last year, before this Society, we reported the effect of urethane in leucemia on experience gained with thirty-five cases. We have now studied its effect in a total of eighty-five cases. Of these leucemias 30 were acute, 3 were

acute monocytic (Naegeli type), 2 were subacute myelocytic, 2 were subacute lymphocytic, 34 were chronic lymphocytic, and 12 were chronic myelocytic. One patient with lymphosarcoma of the skin and one with myeloid metaplasia of the spleen were also observed.

In general, our later observations bear out those previously reported. The most consistent results in using urethane may be expected in those individuals having the chronic myelocytic type. The white blood count may be brought to a normal level and, except for minor fluctuations, may be maintained there as long as a proper dose is given. If the drug is stopped, the count steadily and rapidly rises, only to drop again with institution of urethane therapy. The red blood cell and hemoglobin values are improved during this treatment.

In chronic lymphocytic leucemia the results in over 80 per cent of our series are good as concerns the lowering of the white blood count. It appears that in some individuals larger doses than can be tolerated are needed for producing the desired effect.

The results in the use of urethane in acute leucemia are the least striking. Although one observed occasionally what appears initially to be a dramatic fall in the white count, the patient either succumbs at this level or has an equally sudden rise to even higher than pretreatment levels before death. We have observed in two of our patients a temporary complete bone marrow remission to normal. However, this effect was not maintained.

There is little effect of urethane on the size of the lymph nodes or spleen in lymphocytic leucemia. As a matter of fact, in one patient with chronic lymphocytic leucemia the glands actually increased in size. Our usual daily dose has been from ½ to 3 Gm. orally. Some of our patients have been maintained for eighteen months on the drug, with a total of over 5,000 Gm. being administered. At this dosage level no toxic effects, other than weight loss, drowsiness, and loss of appetite, have been observed in the living. No tissue changes have been observed from the drug as seen at autopsy. Care should be taken in the intravenous administration of urethane as we have observed a fatality when it was given in this manner.

125. CLINICAL OBSERVATIONS WITH METHYL-BIS (B-CHLOROETHYL) AMINE HYDROCHLORIDE

TIBOR J. GREENWALT, M.D., AND ANTHONY J. GRUENINGER, M.D.
MILWAUKEE, WIS.

(INTRODUCED BY MAURICE HARDGROVE, M.D.)

Twenty-four patients have been treated with methyl-bis (B-chloroethyl) amine hydrochloride since November, 1946. The recommended course of treatment consisted of 0.1 mg. per kilogram of body weight given daily for four days.

There were twelve patients with Hodgkin's disease proved by biopsy. Seven of these patients were x-ray resistant. Four of the group have died. The average duration of life after starting therapy was eight months (range, one to thirteen months). The other three are still living two to six months after treatment. In five additional patients nitrogen mustard was used as the initial form of therapy. In three of these the beneficial effect was short-lived. In the other two the results were more satisfactory. One remained in remission for six months and had two courses of the alkyl amine. Later x-ray therapy was instituted. The other has been in complete remission for nine months and has received three courses of therapy. Two of the five have died. Both were given

x-ray therapy after failure of response to mustard. The average duration of disease by history in the six patients who have died was 7.4 years (range, six months to twenty years). The average duration of disease in the six living patients is 1.5 years at present. It appears that the methyl-bis (B-chloroethyl) amine is of value in treating x-ray-resistant cases of Hodgkin's disease. It offers no advantages as an initial form of therapy.

Two patients with lymphosarcoma and two with giant follicle lymphoma were given nitrogen mustard. One of each of these had a partial, unsatisfactory remission. One in each category is still in remission nine months after a single course.

One patient with chronic lymphocytic leucemia, one with chronic myelocytic leucemia, and one with polycythemia vera were treated with partial and brief response.

Two patients with squamous cell bronchiogenic and one with small cell adenocarcinoma of the lung, one with squamous cell carcinoma of the floor of the mouth, and one with malignant myeloma of the chorioid were treated with but slight effect.

126. THE USE OF RADIOARSENIC (As^{76}) IN THE TREATMENT OF LEUCEMIA AND ALLIED DISORDERS OF THE HEMATOPOIETIC TISSUES

MATTHEW H. BLOCK, M.D. (BY INVITATION), LEON O. JACOBSON, M.D., WILLIAM B. NEAL, JR., M.D. (BY INVITATION), AND TAYLOR R. SMITH, M.D. (BY INVITATION), CHICAGO, ILL.

Nineteen patients with tumors of the hematopoietic system have been given As^{76} prepared by pile irradiation of arsenic trioxide and cacodylic acid. Arsenic[76] has a half-life of 26.8 hours and energetic beta and gamma radiations. Doses of As^{76} ranged from 1 to 80 millicuries with 3 to 10 mg. of stable arsenic and were administered intravenously as the trioxide. Preliminary studies indicated that each millicurie was approximately equivalent to one roentgen of total body radiation. In four previously untreated patients with chronic myelocytic leucemia, single injections of this radioisotope produced clinical and hematologic remissions. One of these patients is still in remission after eleven months. However, no significant clinical response was produced by the isotope in two patients with chronic myelocytic leucemia who no longer responded to conventional irradiation therapy. One patient with an acute myelocytic leucemia failed to respond. One patient with a subacute myelocytic leucemia had clinical and hematologic remissions of about two months after each of two injections. However, this latter case required frequent transfusions and an osteomyelitis of the femur failed to heal in spite of chemotherapy and surgery.

Three patients with chronic lymphocytic leucemia treated with less than 30 millicuries were not benefited. One aged male, previously insensitive to nitrogen mustard and needing massive transfusions, responded with a decrease in lymph node enlargement and splenomegaly, an increase in subjective sense of well-being, as well as a depression of the white count for about two months.

Two patients with acute lymphocytic leucemia were treated with 70 and 60 millicuries, respectively. One had a temporary and incomplete remission; in the other, a definite clinical and hematologic remission of approximately eight weeks occurred.

Two patients with polycythemia rubra vera were treated with radioarsenic. A relatively brief remission was produced in one of these patients who was given a dose of 45 millicuries. The other patient received essentially a tracer

dose. Four patients with Hodgkin's disease received less than 13 millicuries of As^{76} each. The clinical effect of the isotope on the course of these four patients was essentially insignificant. Likewise, two patients with multiple myeloma failed to respond favorably to this radioisotope.

The huge liver of a patient with metastases from a carcinoma of the stomach decreased about 5 cm. in size following 80 millicuries of As^{76} in two doses. Nevertheless, his clinical condition continued to deteriorate and he died within two weeks.

127. A METHOD OF FREQUENT ESTIMATIONS OF CIRCULATING PLASMA VOLUME WITH ERYTHROCYTE COUNTS, ADAPTABLE TO LONG-CONTINUED OBSERVATIONS

HENRY E. HAMILTON, M.D. (BY INVITATION), RAYMOND F. SHEETS, M.D. (BY INVITATION), AND ELMER L. DEGOWIN, M.D., IOWA CITY, IOWA

The fluctuations in plasma volume may be studied at will over a period of many days with little inconvenience to the subject. An initial measurement of the plasma volume by Evans blue dye (T-1824) and a coincident erythrocyte count are correlated with subsequent changes in the peripheral erythrocyte counts. The proof depends upon the demonstration of the following facts: (1) With careful attention to the usual technique for counting erythrocytes, values were obtained with a standard deviation of ±68,000 cells per cubic millimeter. (2) Erythrocyte counts on the peripheral blood of normal active adults were found to vary from day to day, sometimes with a range of 1,000,000 cells per cubic millimeter. The variation frequently occurred in cycles of two or three days. (3) In subjects who had received transfusions of blood of heterologous group, the unagglutinable cell counts (donor's blood) varied coincidentally with those of the recipient, in the same direction and in approximately the same magnitude. (4) When estimates of the plasma volume were made from the cell count some days after the original measurement by the dye method, they were found to correspond with a second measurement by the dye within ±5 per cent.

The method consists of an initial measurement of the circulating plasma volume by Evans blue dye coincident with an erythrocyte count. The fluctuations in erythrocyte counts thereafter can be observed over a period of many days and the plasma volume calculated from any single observation by the equation:

$$PV_2 = \frac{C_1 \times PV_1}{C_2}$$

where PV_2 is the calculated plasma volume in milliliters, PV_1 is the plasma volume in milliliters measured by the dye method, C_1 is the erythrocyte count in millions per cubic millimeter in the peripheral blood taken at the time when PV_1 was determined, and C_2 the erythrocyte count in millions per cubic millimeter at the time of the calculation.

The method assumes (1) that the total number of red cells in the body remains the same and (2) that the hematocrit of the peripheral blood bears a constant relationship to the total body hematocrit.

This procedure has been employed to follow the daily fluctuations in plasma volume during normal activity, during hydration and dehydration, and in recipients undergoing blood transfusion. The same method is applicable to the study of many problems in the clinic. Daily erythrocyte counts alone, when accurately performed, should serve as an indication of variations in plasma

volume in patients who are not losing blood and whose bone marrow is functioning normally.

128. THE INFLUENCE OF RUTIN, BENADRYL, AND PROTAMINE ON EXPERIMENTAL PURPURA IN THE GUINEA PIG

FRANK H. BELFUS, M.D. (BY INVITATION), AND FREDERICK W. MADISON, M.D.
MILWAUKEE, WIS.

Thrombocytopenic purpura was produced in eighty guinea pigs by the use of antiplatelet serum according to the method of Bedson. The purpuric animals were divided into four groups of twenty each. One group served as control and received only the previously prepared antiplatelet serum. A second group received rutin, a third group Benadryl, and a fourth group Protamine, simultaneously with the serum. Serial determinations of erythrocytes, platelets, and petechiae were made over a twenty-four hour period following the administration of the serum and the drug. The petechial responses were measured by means of a negative pressure apparatus and the petechiae counted within a standrd area.

The animals of the control group which received only antiplatelet serum exhibited a slight drop in erythrocytes, a marked fall in the platelets, and a sharp rise in petechial formation. The greatest number of petechiae occurred within six hours after the injection of antiplatelet serum which was well in advance of the maximum fall of platelets. When the platelets had reached their lowest numbers the petechial response had subsided.

The second group of animals were given rutin in repeated injections of 10 mg. per kilogram of body weight in addition to the initial dose of antiplatelet serum. These animals exhibited the same trend in platelet depression as the control group. The petechial response of the rutin group was approximately 60 per cent less than that of the control group.

The third group of animals were given the antihistaminic drug, Benadryl, in doses of 10 mg. per kilogram in addition to the antiplatelet serum and a similar platelet depression occurred. However, the petechial response showed reduction of about 40 per cent as compared with the control animals.

The fourth group of animals received protamine in repeated doses of 2 mg. per kilogram in addition to antiplatelet serum. Again the platelet depression followed the usual pattern but the petechial response was markedly diminished. Almost 90 per cent fewer petechiae were produced than in the control group.

Each of the drugs tested therefore showed ability to alter the vascular response (in varying degrees) under the conditions of this experiment but none showed any effect on the thrombocytopenia.

129. EFFECT OF VITAMIN K ON DICUMAROL-INDUCED HYPOPROTHROMBINEMIA IN RATS

E. J. BOYD, M.D. (BY INVITATION), AND E. D. WARNER, M.D.
IOWA CITY, IOWA

Most of the reports dealing with the effect of vitamin K on Dicumarol-induced hypoprothrombinemia have been based on one or another of the one-stage methods for prothrombin estimation. It has been shown that these methods depend not only on the concentration of prothrombin, but also on the rate of conversion of prothrombin to thrombin. The two-stage method of prothrombin determination separates the conversion phase from the clotting phase and thereby largely eliminates the rate of conversion of prothrombin to

thrombin as a factor in prothrombin measurement. For this reason, it seemed pertinent to study the effect of vitamin K on Dicumarol-induced hypoprothrombinemia by the two-stage prothrombin method.

Albino rats were made hypoprothrombinemic by the administration of Dicumarol. To control the dosage better, the Dicumarol was given by stomach tube. Vitamin K was given in daily doses as menadione in oil by stomach tube, or as Hykinone by intraperitoneal injection. Doses as large as 50 mg. of menadione and 4 c.c. Hykinone (1 c.c. equivalent to 2.5 mg. menadione) per day were used. The data obtained by the two-stage prothrombin determination indicate that (1) rats tend to develop a tolerance to Dicumarol, (2) massive doses of vitamin K in the form of menadione, or Hykinone, have no definite effect on Dicumarol-induced hypoprothrombinemia in rats, from either the prophylactic or the curative standpoint.

130. THE USE OF INTRAMUSCULAR HEPARIN FOR ANTICOAGULANT THERAPY

GEZA DE TAKATS, M.D., AND EVELYN M. ROGERS, R.N. (BY INVITATION)
CHICAGO, ILL.

In a series of 100 patients, subcutaneous and intramuscular injections of heparin were given to maintain a prolonged coagulation time for prophylactic or therapeutic purposes. The solutions used were 1 and 10 per cent aqueous heparin and a preparation which uses gelatin dextrose and vasoconstrictors to delay absorption.

After considerable trial with various dosage and timing, the following tentative schedule has been developed:

The patient first is given 1 c.c. of 1 per cent heparin intravenously to determine his heparin tolerance. The coagulation time is determined before and only once, ten minutes, after the injection. If there is a 100 per cent increase in coagulation time following the injection, he is regarded as having a normal response and is given 5 c.c. of heparin intravenously. One hour following this injection, which usually raises coagulation time to about 15 minutes by the venous method, the first intramuscular injection is given with the heparin glycerin mixture. In from twelve to sixteen hours this is repeated; the coagulation time by the capillary method is always done before the next injection to avoid a stepladder type of effect.

There has been no reaction to the intramuscular injections either in the form of hematoma or induration, and the coagulation times have shown a stable level which was hitherto impossible to obtain even by the continuous intravenous drip.

131. THROMBOCYTOPENIA SECONDARY TO INFECTIOUS MONONUCLEOSIS

RICHARD M. ANGLE, M.D. (BY INVITATION), AND HOWARD L. ALT, M.D.
CHICAGO, ILL.

Thrombocytopenic purpura is a rare complication of infectious mononucleosis. A report is made of a 19-year-old white female student nurse who developed petechiae, ecchymoses, and menorrhagia during the course of infectious mononucleosis. The platelet count fell to 10,000 per cubic millimeter. Spontaneous recovery accompanied convalescence from the infectious mononucleosis. The patient has remained well with a normal platelet count and no further bleeding episodes.

A review of the literature reveals only two identical cases. In two other reported instances there appeared to be a pre-existing bleeding diathesis which was aggravated by the infectious mononucleosis. In other possible cases the diagnosis of infectious mononucleosis was never definitely established or insufficient details were reported to permit comparison.

Although the platelet count is said to be normal in infectious mononucleosis, no report of serial counts in this disease was found. We have determined the number of platelets at frequent intervals in seven consecutive cases of infectious mononucleosis. In five of the seven patients the platelet count was below the lower limit of normal during the first week of the disease. In one case the platelets were just above the lower limit of normal, and in another, initial counts were not made until the second week of the disease. During the second week of the illness a mild thrombocytosis varying from one and one-half to five times the lowest count occurred in six patients. This was followed by a gradual return to normal values. The case, studied first in the second week of the disease, showed a mild thrombocytosis which subsequently returned to normal. In four of the seven patients the spleen was palpable, and abnormal liver function was present in all.

The possibility that thrombocytopenic purpura in infectious mononucleosis may result from a similar but exaggerated depression of the blood platelets is suggested.

The possible roles of infection and hypersplenism as a cause of the thrombocytopenia will be discussed.

132. THE EFFECT OF DIGITALIS ON THE COAGULABILITY OF BLOOD

JOHN HYSLOP FLINN, M.D., ROCHESTER, MINN.

(INTRODUCED BY HOWARD B. BURCHELL, M.D.)

The high incidence of thromboembolic complications in cases of congestive heart failure is generally recognized. In the past five years several reports have appeared in the literature which implicated digitalis as a contributing factor to intravascular coagulation of the blood. Other reports have subsequently appeared which seem to deny this implication. The present study was undertaken in an attempt to determine whether certain cardiac glucosides do change the coagulability of the blood and, if so, how these changes can be measured. Because of the controversial reports, it seemed evident that an investigation which would include both animal and clinical research and would consist of a study of blood coagulation by several methods before and during digitalization was indicated.

The problem was first investigated in the physiology laboratories of the Mayo Clinic and rabbits and rats were used. The study was completed in the Worrall Hospital, Rochester, Minn., with patients on the cardiac services of the Mayo Clinic. Studies of coagulation included the Lee-White coagulation time, prothrombin times of whole and diluted plasma, and heparin-retarded coagulation time.

Results of the investigation show that lanatosid-C and digitoxin administered to normal rabbits and rats in doses simulating therapeutic use of the drugs have a definite accelerating effect upon coagulation of the blood as measured by the Lee-White coagulation time tests; in rabbits the in vitro heparin-retarded coagulation time test showed a similar effect on coagulation of the blood. No effect was noted on the prothrombin time of whole or diluted plasma. In the normal rabbit relatively large doses of lanatosid-C appear to have an antagonistic effect to Dicumarol.

The blood of clinically digitalized patients was not changed from that of normal subjects as measured by the Lee-White coagulation time tests or by the prothrombin time studies of whole or diluted plasma. However, digitalization appeared to diminish the anticoagulant effect of heparin in the human being.

These results seem to indicate that digitalis may be a factor in promoting intravascular coagulation of blood.

EDITORIAL ANNOUNCEMENT

On January 1, 1949, Doctor Clayton G. Loosli will become Editor of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE. After that date, all correspondence relating to editorial matters should be addressed to him at the University of Chicago, Department of Medicine.

For the past two years, this Journal has been published under the editorial direction of the Central Society for Clinical Research. It seems appropriate now to announce the way in which the Editor and the members of the Board of Editors are selected. The Editor is appointed by the Council of the Central Society for a term of three years; he may be appointed for a second three-year term, but may not serve consecutively for longer than six years. When the Editorship is to be vacated for any reason, the Board of Editors recommends a successor from the Society's membership to the Council. The Council may accept this recommendation or may make its own appointment. Doctor Loosli represents the unanimous and enthusiastic choice of the Council, the Board of Editors, and the retiring Editor.

Members of the Board of Editors are appointed by the Editor to serve for a three-year period. Each member is eligible for reappointment to a second, but not to a third consecutive term. Appointments are staggered so that four or five terminate at the end of each calendar year. Members of the Board are selected from among the active, emeritus, and adjunct membership of the Central Society for Clinical Research.

The retiring Editor wishes to express publicly his sincere appreciation: first, to all members of the various Editorial Boards who have served with him during the past five years; second, to the many clinical investigators who have supported the Journal by sending their manuscripts to it; and third, to the editorial department of The C. V. Mosby Company for constant, wholehearted cooperation.

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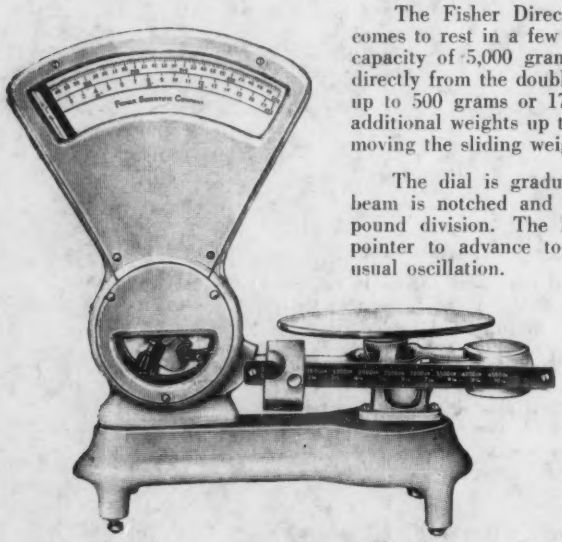

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